The Role of Mitogen-Activated Protein Kinases in Inflammasome Activation

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von

Gabriele Fenini

von

Massagno, TI

Promotionskommission
Prof. Dr. Burkhard Becher (Vorsitz)
Prof. Dr. Lars E. French (Leitung der Dissertation)
Prof. Dr. Onur Boyman

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To mom and dad

ZUSAMMENFASSUNG

Die Erkennung evolutionär konservierter mikrobieller Strukturen und Gefahrenmoleküle, die von gestressten oder sterbenden Zellen freigesetzt werden, ist ein wichtiger Schritt bei der Einleitung einer Immunantwort. Die Erkennung von Pathogen- oder Gefahr-assoziierten molekularen Mustern (*pathogen--associated molecular patterns,* PAMPs) oder *danger- associated molecular patterns,* DAMPs) durch Mustererkennungsrezeptoren (PRRs, *pattern recognition receptor*) kann Signalkaskaden aktivieren, die zur Expression von Genen führen. Diese Gene kodieren Faktoren, die in Immunantworten und in ihrer Regulierung involviert sind. Die PRRs Subfamilie von NOD-ähnlichen Rezeptoren (*NOD-like receptors,* NLRs) besteht aus zytosolischen Sensoren, die bei der Erkennung von verwandten Molekülen oligomerisieren können und anschließend das Enzym Caspase-1 über das Protein Apoptose-assoziierte speckähnliche Protein, das eine CARD enthält (*apoptosis-associated speck-like protein containing a CARD,* ASC), rekrutieren und aktivieren. Diese Multiproteinkomplexe, Inflammasome genannte, wurden erstmals im Jahr 2002 beschrieben. Ihre Aktivierung führt zu einer Caspase-1-abhängigen Verarbeitung und Sekretion der proinflammatorischen Zytokine Interleukin (IL)-1β und IL-18. Mehrere autoinflammatorische Krankheiten wie Cryopyrin-assoziierte periodische Syndrome (CAPS) und das familiäre Mittelmeerfieber (*Familial Mediterranean Fever*, FMF) wurden mit Mutationen von Genen assoziiert, die für Inflammasom-Komponenten kodieren. Die Inflammasome wurden intensiv in myeloiden Zellen wie Makrophagen und dendritischen Zellen untersucht. Im Gegensatz dazu wurde die Regulierung der Inflammasome in Zellen der äussersten Hautschicht, den Keratinozyten, noch nicht genauer untersucht. Tatsächlich sind Keratinozyten auch immunologisch aktive Zellen, die funktionelle PRRs einschließlich NLRs exprimieren und in der Lage sind, aktives IL-1β zu sezernieren. Mitogen-aktivierte Proteinkinasen (*mitogen-activated protein kinases*, MAPKs) sind eine Familie von evolutionär konservierten Kinasen, einschliesslich der extrazelluläre signalregulierte Kinase (ERK), der Jun N-terminale Kinase (JNK) und der p38 MAPK. Sie sind an grossen physiologischen Signalwegen beteiligt, indem sie die Gentranskription, die Proteinbiosynthese und die Kontrolle des Zellzyklus, der Differenzierung und der Apoptose regulieren. Mehrere Studien haben die Kinaseaktivität mit der Kontrolle und Aktivierung von Inflammasomen in myeloiden Zellen in Zusammenhang gebracht.

In dieser Dissertation haben wir die Rolle von MAPKs, insbesondere von p38 und JNK, bei der Kontrolle und Aktivierung von Inflammasomen in menschlichen primären Keratinozyten und myeloiden Zellen untersucht. Mit selektiven kleinen Molekül-Inhibitoren gegen MAPKs konnten wir zeigen, dass p38 die Inflammasomaktivierung in Keratinozyten während und nach Bestrahlung mit ultraviolettem Licht B (UVB) und Behandlung mit Nigericin, einem mikrobiellen Toxin, reguliert. Unter Verwendung von RNA-Silencing und CRISPR / Cas9-basierter Deletion zeigten wir, dass die

p38α- und p38δ-Isoformen kritische Regler von ASC-Oligomerisierung, Inflammasomaktivierung und IL-1β/IL-18-Sekretion in Keratinozyten sind. Ausserdem führte die Überexpression des aktiven p38α-Isoform zu einer spontanen Sekretion von IL-1β. Weiterhin untersuchten wir die Beteiligung von p38 an der Phosphorylierung des Inflammasom-Adapters ASC, konnten jedoch keine Phosphorylierung von ASC in Keratinozyten nachweisen. Interessanterweise zeigten unsere Daten, dass Nigericin, welches zuvor als NLRP3-Inflammasom-Aktivator beschrieben wurde, in der Lage ist, die IL-1β-Sekretion in Keratinozyten in einer NLRP1-abhängigen Weise zu induzieren. Ähnlich wie bei p38α und δ konnten wir durch shRNA-Interferenz in menschlichen THP-1-Zellen und mit JNK-Knockout-Tieren zeigen, dass die JNK1-Isoform eine dominierende Rolle bei der Regulation des Inflammasoms in myeloiden Zellen einnimmt. Außerdem führte die Überexpression von aktivem JNK in myeloiden Zellen zu einer erhöhten IL-1β-Sekretion nach Behandlung mit Inflammasomaktivatoren. Wir konnten eine Strategie entwickeln, um die Genexpression in primären Keratinozyten durch die CRISPR/Cas9-Technologie effizient zu erzielen. Die Zielzellen konnten sich *in vitro* vermehren und differenzieren und zeigten ähnliche Inflammasomaktivität wie in Wildtyp-Zellen.

Zusammenfassend erweitern unsere Erkenntnisse das Spektrum der Kinasen, die die Inflammasomaktivierung regulieren. Wir konnten zeigen, dass die an diesem Prozess beteiligten MAPKs eine Zelltypspezifität aufweist.

SUMMARY

The recognition of evolutionarily conserved microbial structures and danger molecules released by stressed or dying cells is an important step in the initiation of an immune response. Recognition of pathogen- or danger-associated molecular patterns (PAMPs or DAMPs, respectively) by pattern recognition receptors (PRRs) can activate signaling cascades that result in the expression of genes encoding factors involved in immune responses and in their regulation. The PRRs subfamily of NOD-like receptors (NLRs) consists of cytosolic sensors that are able to oligomerize upon sensing cognate molecules and subsequently recruit and activate inflammatory caspase-1 via the adaptor protein, known as apoptosis-associated speck-like protein containing a CARD (ASC). These complexes, named inflammasomes, were first described in 2002. Their activation results in caspase-1-dependent processing and secretion of the proinflammatory cytokines interleukin (IL)-1β and IL-18. Several autoinflammatory disorders such as cryopyrin-associated periodic syndromes (CAPS) and Familial Mediterranean Fever (FMF) have been associated with mutations of genes encoding inflammasome components. Inflammasomes have been extensively studied in myeloid cells such as macrophages and dendritic cells. In contrast, little is known about inflammasome regulation in keratinocytes, the cells that constitute the outermost layer of the skin. Indeed, keratinocytes are also immunologically active cells expressing functional PRRs including NLRs and are able to secrete active IL-1β. Mitogen-activated protein kinases (MAPKs) are a family of evolutionary conserved kinases that include extracellular signal-regulated kinase (ERK), Jun N-terminal kinase (JNK) and p38 MAPK. They are involved in major physiological pathways such as cell cycle, differentiation and apoptosis by regulating gene transcription and protein biosynthesis. Several studies have linked kinase activity with the control and activation of inflammasomes in myeloid cells.

In this thesis, we investigated the role of MAPKs, specifically p38 and JNK, in the regulation of inflammasome activation in human primary keratinocytes (HPKs) and myeloid cells. Using selective small molecule inhibitors against MAPKs we could show that p38 regulates inflammasome activation in HPKs upon ultraviolet B (UVB) irradiation and treatment with nigericin, a microbial toxin known to activate the inflammasome. Using RNA silencing and CRISPR/Cas9-based deletion we showed that the p38α and p38δ isoforms are critical regulators of ASC oligomerization, inflammasome activation and IL-1β/IL-18 secretion in keratinocytes. Moreover, overexpression of active p38α resulted in spontaneous processing of IL-1β. Furthermore, we were able to show that p38 is not involved in the phosphorylation of ASC in HPKs. Interestingly, our data also suggest that nigericin, previously described as an NLRP3 inflammasome activator, is able to induce IL-1β secretion in keratinocytes in an NLRP1-dependent manner. Similarly to p38α and δ, we could show by shRNA interference in human THP-1 cells and with JNK-knockout animals that the JNK1 isoform has a predominant role in the regulation of the inflammasome in cells of myeloid origin. Moreover, overexpression of active JNK in

myeloid cells also resulted in increased IL-1β secretion upon exposure to inflammasome activators. Finally, we developed a strategy to efficiently target gene expression in HPKs by CRISPR/Cas9 technology. Targeted cells could proliferate and differentiate *in vitro* and were successfully used to study inflammasome activity.

Taken together, our findings further expand the spectrum of kinases that regulate inflammasome activation and suggest that the nature of the MAPK involved in this process exhibits a certain cell type specificity.

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CHAPTER 1: INTRODUCTION

Part of this introduction is adapted from the review article: **Gabriele Fenini**, Emmanuel Contassot and Lars E. French, "Potential of IL-1, IL-18 and inflammasome inhibition for the treatment of inflammatory skin diseases", *Frontiers in Pharmacology* (Fenini et al., 2017).

1.1 THE INNATE IMMUNE SYSTEM

Every organism must face the challenge of protecting itself from invading pathogens including bacteria, viruses and fungi but also from environmental insults such as radiations or chemicals.

Such a protection of the host from pathogen invasion or any environmental insult is achieved by several mechanisms. First, anatomic barriers like the skin and mucosa physically impair the entrance of microorganism; then, in a process known as humoral innate immunity, soluble proteins such as antimicrobial enzymes or peptides and the complement system target pathogens in a non-specific manner. If this process fails, innate immune cells get then activated upon recognition of conserved molecular patterns derived from the invaders and recruit more innate immune cells to the infection site by secreting effector molecules such as cytokines and chemokines. This process is known as inflammatory response or inflammation. The third phase of host defense consists in a specific response of the adaptive immune system against the pathogen involving B and T cells. The maturation of cytotoxic T cells and mature B cells requires the support from the innate system through antigen presentation, costimulatory signaling and cytokine release. In contrast to the immediate response of the innate immune system, adaptive immunity requires few days to develop but has the advantage to be much more specific and diverse and to display immunological memory (Figure 1) (Murphy and Weaver, 2016).

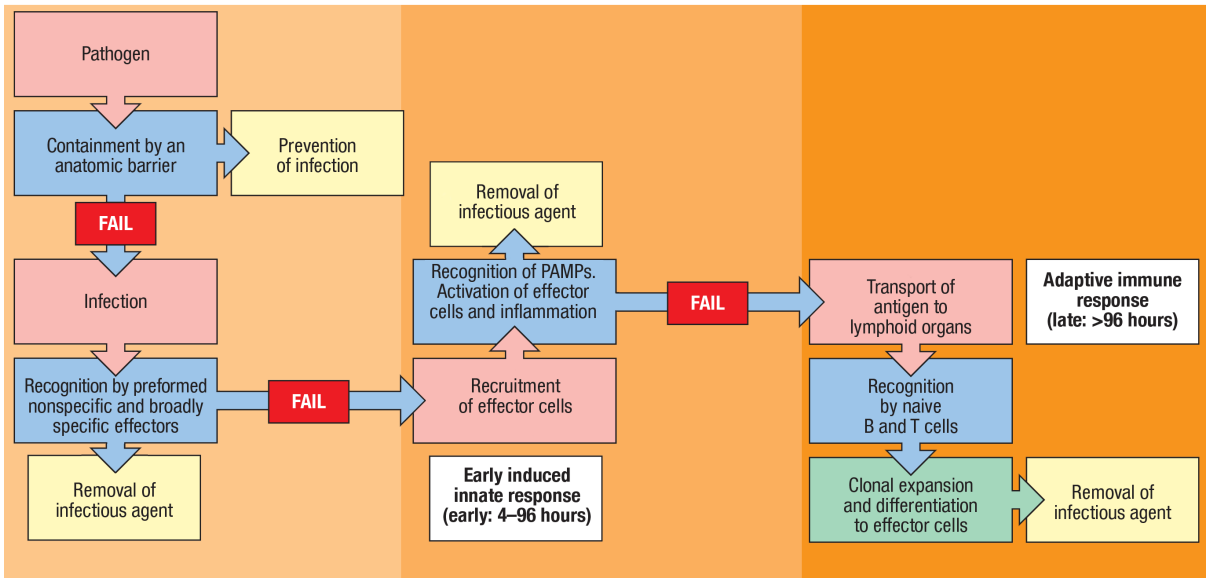

Figure 1 Phases of immune responses to infection

The immune response to infection includes three phases: an immediate innate phase, an early induced innate response and a late adaptive immune response. See text for further description. Adapted from (Murphy and Weaver, 2016).

Cells of the innate immune system include monocytes and macrophages (Mϕs), dendritic cells (DCs), granulocytes (neutrophils, eosinophils, basophils and mast cells), the recently identified innate lymphoid cells (ILCs) including natural killer (NK) cells but also epithelial cells.

Epithelial cells play a fundamental role in innate immunity. They compose the skin and the mucosa of the oral, respiratory, gastrointestinal and urogenital tracts. Epithelia constitute a physical barrier between the host and the external world, thus providing a mechanical protection. Moreover, epithelial cells release soluble antimicrobial peptides (AMPs) and enzymes such as defensins, cathelicidins, lamellar bodies and pepsins which chemically target pathogens (Afshar and Gallo, 2013; Murphy and Weaver, 2016). Also, epithelia exhibit microbiological immunity due to the competition for the same ecological niche between the normal microbiota and invading microbes (Kamada et al., 2013; Belkaid and Hand, 2014).

Neutrophils are the most abundant immune cells in humans. These highly motile granulocytes can rapidly migrate to the site of infection to release soluble AMPs by degranulation or phagocytose pathogens (Kruger et al., 2015). Moreover, they can form neutrophil extracellular traps (NETs) by releasing meshwork of chromatin fibers and AMPs to capture and kill bacteria or fungi (Brinkmann et al., 2004; Jorgensen et al., 2017). Other granulocytes, that include basophils, eosinophils and mast cells, are important players in allergic inflammation through the IgE-mediated release of inflammatory mediators like heparin and histamine (Stone et al., 2010).

ILCs are important innate immune cells essential for several physiological processes ranging from metabolic homeostasis to tissue remodeling but also regulation of autoimmune inflammation and resistance to pathogens (Klose and Artis, 2016). ILCs arise from common lymphoid progenitor cells like T and B lymphocytes and they all have a counterpart for each T cell subset, excluding regulatory T cells (Zook and Kee, 2016). NK cells can kill directly target cells. Direct killing of malignant or infected cells is achieved by antibody-dependent cellular cytotoxicity, where NK cells recognize, via surface Fc receptors, opsonized target cells and consequently release cytotoxic granules containing perforin and granzyme (Voskoboinik et al., 2015). Another mechanism involves the killing mediated by death receptors of cells that exhibit an altered major histocompatibility complex (MHC) class I expression (missing-self recognition) (Karre, 2002). NK cells and ILC1s are characterized by their IFNγ production; ILC1 cells play an important role in the intracellular immunity by accelerating macrophage phagocytosis (Moro and Koyasu, 2015). ILC2 cells are involved in mucosal and barrier immunity: secretion of IL-5 and IL-13 favors the elimination of parasites inducing goblet cell hyperplasia, mucin production and the recruitment of eosinophils. ILC3s play a crucial in the resistance to bacterial and fungal infections by secreting IL-22 and IL-17 and recruiting neutrophils.

DCs and **Mϕs** are professional antigen-presenting cells (APCs), able to present antigens on MHC class II (MHC-II) to $CD4^+$ helper T cells. The main feature of macrophages is their capacity to engulf large particles but also pathogenic microorganisms and cell debris, a form of endocytosis called phagocytosis. DCs are also able phagocytose, however their phagosomal degradation is milder and, therefore, allows

the conservation of enhanced antigenic information (Savina and Amigorena, 2007). Next to phagocytosis, degranulation and release of AMPs, innate immune cells can counteract invading pathogens by secreting cytokines and chemokines to initiate an inflammatory response. This reaction is driven by the sensing of pathogens or danger molecules by receptors with limited specificity but able to recognize repeated chemical structures, the so-called patter recognition receptors (PRRs).

PATTERN RECOGNITION RECEPTORS

In 1989, Janeway proposed that innate immune cells were able to initiate an immune response with the recognition of evolutionarily conserved microbial structures termed pathogen-associated molecular patterns (PAMPs) via germ-line encoded PRRs (Janeway, 1989). Five years later, Matzinger suggested that danger molecules released by stressed or dying cells rather than PAMPs are able to activate the immune system also via PRRs (Matzinger, 1994). These danger molecules, which include extracellular DNA, RNA and ATP, the DNA-binding alarmin high-mobility group protein B1 (HMGB1) and heat-shock proteins, were named by similarity danger-associated molecular patterns (DAMPs).

PRRs include C-type lectin receptors (CLRs), Toll-like receptors (TLRs), retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), AIM2-like receptors (ALRs) and, partially, the complement system[a]. Such PRRs have different localizations and ligands (Table 1). For example: the complement system acts as an extracellular sensor for host antibodies or conserved pathogen motives (carbohydrates) via mannose-binding lectins, a type of CLRs (Dunkelberger and Song, 2010).

[a] The complement system is mainly activated by interaction with the Fc region of immunoglobulins bound to antigens (classic pathway) or to mannose-binding lectins (lectin pathway). Additionally, the C3 components of the complement system can covalently bind the surface of pathogens in an antibody independent way, thus having similar characteristic to PRRs (Serruto et al., 2010).

Type	Name	Ligand	Localization	Function	Adaptor/ partner
TLR	TLR1	peptidoglycan, lipoproteins (with TLR2)	cell surface	signaling	MyD88/Mal
	TLR2	see TLR1 and TLR6	cell surface	signaling	MyD88/Mal
	TLR3	dsRNA, poly I:C	endosomal	signaling	TRIF
	TLR4	LPS, HSPs, β-defensin, lipoprotein, nickel	cell surface endosomal	signaling	MyD88/TRIF TRIF/TRAM
	TLR5	flagellin, profilin	cell surface	signaling	MyD88
	TLR6	lipoproteins (with TLR2)	cell surface	signaling	MyD88/Mal
	TLR7	ssRNA (viral), small synthetic compounds	endosomal	signaling	MyD88
	TLR8	ssRNA (viral)	endosomal	signaling	MyD88
	TLR9	CpG/DNA	endosomal	signaling	MyD88
	TLR10	unknown	cell surface	signaling	
NRL	NOD1	iE-DAP	cytosolic, endosomal membrane	signaling	RIPK2
	NOD2	MDP, ssRNA (viral)	cytosolic, endosomal membrane	signaling	RIPK2, MAVS
	NLRP1	MDP	cytosolic	inflammasome	ASC
	NLRP3	oxDNA	cytosolic	inflammasome	ASC
	NLRC4	flagellin, T3SS	cytosolic	inflammasome	NAIP, ASC
	NAIP	flagellin, T3SS	cytosolic	inflammasome	NLRC4
	pyrin		cytosolic	inflammasome	
ALR	AIM2	dsDNA	cytosolic	inflammasome	ASC
	IFI16	dsDNA	cytosolic, nuclear	inflammasome, signaling	ASC, STING
RLR	RIG-I	ssRNA, dsRNA	cytosolic	signaling	MAVS
	MDA5	dsRNA	cytosolic	signaling	MAVS
CLR	mannose receptor	mannose, fucose, GlcNAc	cell surface	endocytic	-
	dectin-1	β-glucan	cell surface	endocytic, signaling	Syk
	mincle	α-mannose, TDM, SAP130	cell surface	endocytic, signaling	FcRγ/Syk

Table 1 Selected pattern recognition receptors, their ligands, localizations and functions

Abbreviations: iE-DAP, γD-glutamyl-meso-diaminopimelic acid; MDP, muramyl dipeptide; T3SS, bacterial type III secretion system; GlcNAc, N-Acetylglusamine; TDM, trehalose-6'6'-dimycolate (glycolipid); SAP130, spliceosome-associated protein 130.

Toll-like receptors (TLRs), are found on cellular membrane and endosomes and bind to a variety of molecules including nucleic acids (TLR3, TLR7, TLR8, TLR9, TLR13[a]), small proteins (TLR2, TLR4, TLR5, TLR11[a], TLR12[a]), lipopeptides and lipoproteins (TLR1, TLR2, TLR6), glycolipids (TRL2, TLR4) and small drugs (TLR4, TLR7) (Leifer and Medvedev, 2016). TLRs are transmembrane glycoproteins characterized by an extracellular domain responsible for ligand recognition, a

[a] *Tlr11*, *12* and *13* genes are functionally expressed only in mice; *TLR11* is found in human as pseudogene whereas genes encoding TLR12 and TLR13 are completely absent (Yarovinsky, 2014). *Tlr10* is a pseudogene in mice.

transmembrane domain, and an intracellular signaling Toll/Interleukin-1 receptor (TIR) domain. Their ligand specificity depends on the variability of leucine-rich repeat (LRR) modules in the horseshoe-like extracellular structure (Kedzierski et al., 2004). Based on the localization of the receptor, distinct adaptor sets are recruited and different signaling pathway are activated (Yamamoto et al., 2002); for example, endosomal TLR3, TLR7, TLR8 and TLR9 induce the production of type I interferons. Activation of TLR signaling requires homo- or hetero-dimerization of the receptor and recruitment of the adaptors MyD88/Mal and TRIF/TRAM via TIR domain interaction. This activates IL-1R-activating kinases (IRAKs) and tumor necrosis factor (TNF)-receptor-associated factors (TRAFs) which in turn trigger p38 and JNK mitogen-associated protein kinases (MAPKs), the transcription factor NF-κB and the interferon regulatory factors (IRFs) (Gay and Gangloff, 2007) (Figure 2). The sensing of peptidoglycans and lipoproteins by TRL1 and TLR6 involves heterodimerization with TLR2 and requires interaction with the co-receptors CD14 and CD36, respectively (Hoebe et al., 2005; Manukyan et al., 2005). LPS recognition by TLR4 requires the formation of a complex consisting of LPS-binding protein, CD14, and MD-2 (da Silva Correia et al., 2001), whereas flagellin causes homodimerization of TLR5 (Hayashi et al., 2001). Altogether, activation of these receptors culminates with the activation of the MyD88 pathway with the expression of many proinflammatory genes. Interestingly, TLR4 can also be found in endosomal complex where its activation drives a late wave of NF-κB activation and type I IFN production via TRAM/TRIM signaling (Yamamoto et al., 2003). The endosomal TLR3 and TLR9 sense dsDNA and unmethylated CpG DNA motifs, respectively, while TLR7 and TLR8 recognize ssRNA. Restriction of these receptors to this subcellular location is a strategy to increase their chemical specificity since self-nucleic acids are unlikely to be present (Brubaker et al., 2015). TLR10 is currently the only receptor of this family without a known ligand but it has been shown that it possesses anti-inflammatory properties by inducing IL-1 receptor antagonist (IL-1Ra) or the formation of inhibitory heterodimer with TLR2 (Oosting et al., 2014).

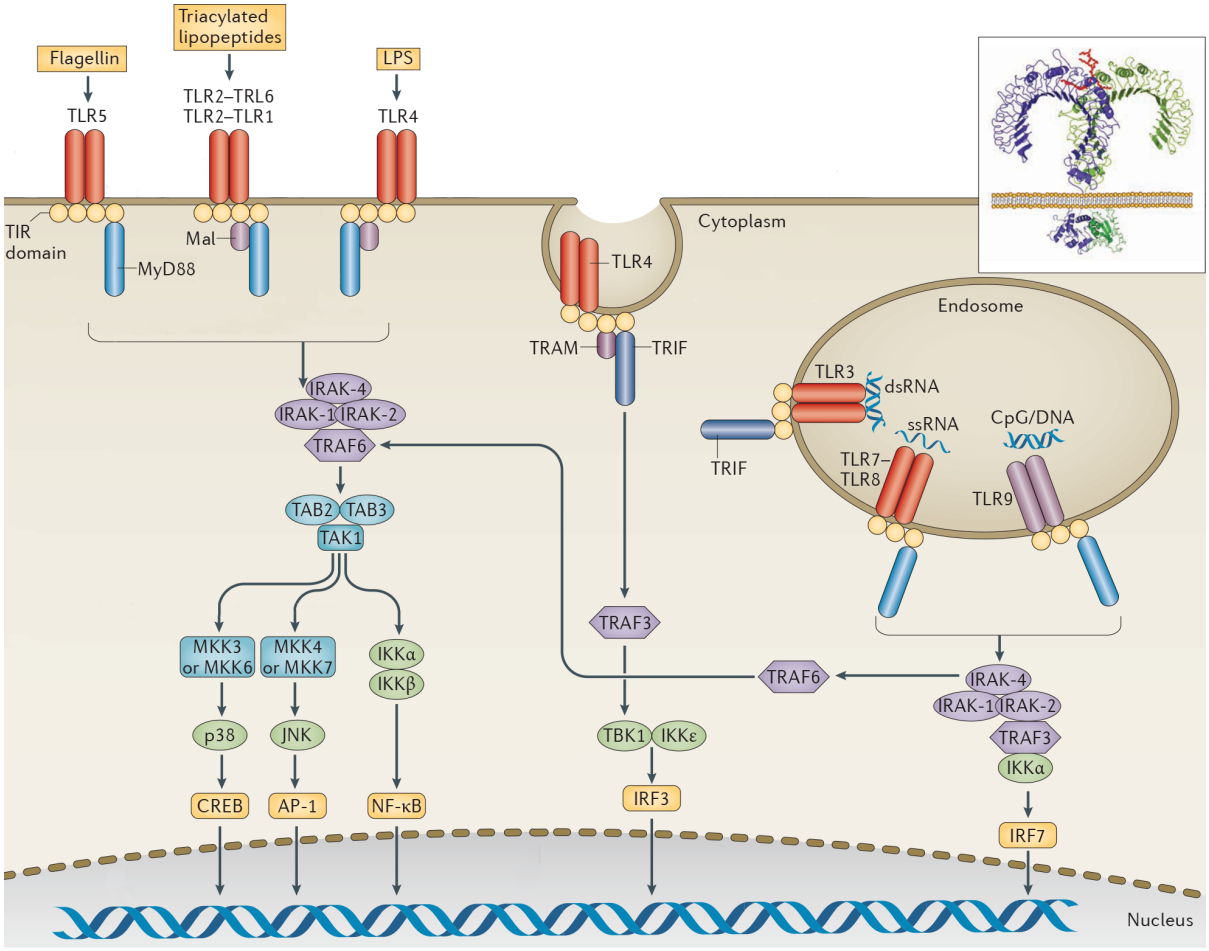

Figure 2 Localization and signaling pathways of human TLRs

Cell surface TLR1, TLR2, TLR4, TLR5 and TLR6 signal through MyD88 to activate JNK and p38 mitogen-associated protein kinases (MAPKs) and NF-κB. Endosomal TLR3, TLR7, TLR8, TLR9 and internalized TLR4 can activate the production of type I interferons via TRIF and Myd88. Upper right corner: crystal structure of the TLR1:TLR2 heterodimer with characteristic horseshoe-like extracellular domains. Adapted from (Kerur et al., 2011) and (Murphy and Weaver, 2016).

C-type lectins are a group of heterogeneous soluble or membrane-bound molecules that play various functions. The mannose-binding protein (MBP) and other collectins do not possess a proper signaling potential but act as opsonins activating the complement system via the lectin pathway. Others, such as selectins, play an important role in cell-adhesion or initiate inflammatory responses (Cummings and McEver, 2009). **C-type lectin receptors (CLRs)** are localized either at the cell surface or in endosomes and primarily bind to carbohydrates (mannose, fucose, GlcNAc, β-1,3-glucan) in a Ca^{2+}-dependent manner but the recognition of proteins, lipids and inorganic compounds like $CaCO_3$ has also been reported (Zelensky and Gready, 2005). They promote phagocytosis (mannose receptor, DC-SIGN, ASGPR) but are also able to induce gene expression and modulate TLR signaling (Geijtenbeek and Gringhuis, 2009). Dectin-1 recognizes β-glucan, a cell wall component of bacteria, fungi and yeasts (Brown et al., 2003) and initiates a Syk-dependent signaling cascade that activate NF-κB via protein kinase C δ (PKCδ) and CARD9/Bcl-10/MALT-1 signalosome (Strasser et al., 2012). Mincle recognizes the fungal cell wall component α-mannose but also spliceosome-associated protein 130 (SAP130), a

DAMP released by necrotic cells (Yamasaki et al., 2008) and initiates, through its Fc receptor-γ (FcRγ) domain, a downstream signaling pathways similar to that of dectin-1.

Members of **RIG-I-like receptors (RLRs)** include RIG-I, melanoma differentiation gene 5 (MDA5) and the co-receptor laboratory of genetics and physiology 2 (LGP2). They are specialized in the host defense against RNA viral infection (Reikine et al., 2014). They discriminate endogenous from foreign RNA by recognizing distinct viral features such as long double-stranded RNA (dsRNA), poly-uridine regions and 5′-triphosphate RNA (Kato et al., 2008; Saito et al., 2008). Upon RNA interaction, RLRs translocate to the mitochondrial membrane where they activate the mitochondrial antiviral-signaling protein (MAVS). Then, MAVSs polymerize resulting in the activation of the transcription factors NF-κB, IRF3, and IRF7 controlling the expression proinflammatory cytokines, IFN and IFN-stimulated genes.

The **AIM2-like receptor (ALR)** family includes AIM2 and IFI16. They are involved in the detection of intracellular DNA via their DNA-binding HIN-200 domain (Unterholzner et al., 2010). ALRs also possess a pyrin and HIN domain-containing protein (PYHIN or HIN-200) domain which allow them to interact with other protein to form an inflammasome (discussed below). Additionally, IFI16 is involved in viral DNA detection within the nucleus with consequential activation of the STING (stimulator of IFN genes)-dependent signaling (Kerur et al., 2011).

NOD-like receptors (NLRs) constitute an expanding family of cytosolic receptors able to detect a variety of molecules. They are composed of several domains: the central NOD or nucleotide-binding domain (NBD) that includes a NTPase NACHT domain controlling self-oligomerization, and the leucine-rich repeat (LRR) domain involved in ligand sensing (Schroder and Tschopp, 2010). On their N-terminal extremity, NLRs have either a pyrin domain (PYD), a caspase-recruitment domain (CARD) or a baculoviral inhibition of apoptosis protein repeat domain (BIR) and are consequently named NLRPs, NRLCs or NAIPs, respectively (Figure 3). Other NLRs include NOD1 and NOD2, which share structural similarity with NLRCs, hence also referred as NLRC1 and NLRC2, but have a shorter LRR domain and a single or tandem N-terminal CARD, respectively (Philpott et al., 2014). They are found in the cytosol but they translocate to the endosomal membrane where they detect bacterial cell membrane components such as γD-glutamyl-meso-diaminopimelic acid (iE-DAP), muramyl dipeptide (MDP) mediating NF-κB and MAPKs activation via recruitment of the signaling kinase RIPK2 (Bonham and Kagan, 2014). They are also involved in the regulation of mesenchymal stem cells differentiation increasing ERK MAPKs signaling and autophagy, a process allowing the degradation of unwanted or dysfunctional cellular components (Kim et al., 2010; Travassos et al., 2010). Furthermore, NOD2 is also involved in the recognition of viral RNA and the production of IFNβ via IRF-3 (Sabbah et al., 2009).

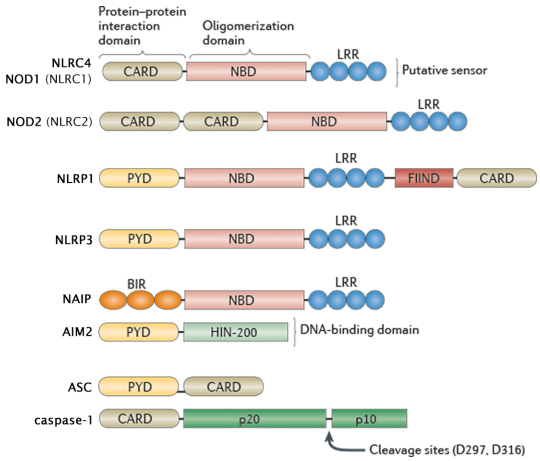

Figure 3 Structures of common NLRs and inflammasome components

NLRs have in common the central nucleotide-binding domain (NBD) and the C-terminal leucine-rich repeat (LRR) domain and differ by their N-terminal domains: NLRP1 and NLRP3 have a pyrin domain (PYD) whereas NLRC4, NOD1 and NOD2 have a caspase-recruitment domain (CARD) and NAIP has a baculoviral inhibition of apoptosis protein repeat domain (BIR). Moreover, NLPR1 has a CARD on its C-terminal. AIM2 has a double-stranded DNA recognizing domain (HIN-200). NLRP3 and AIM2 require the adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC) to recruit the inflammatory caspase-1. Adapted from (Walsh et al., 2014).

THE INFLAMMASOMES

In 2002, the group of Prof. Tschopp described a multiprotein complex able to oligomerize and activate inflammatory caspases leading to the processing of IL-1β and IL-18 (Martinon et al., 2002). This complex was named NLR PYD-containing protein 1 or **NLRP1 inflammasome** and was shown to contain the scaffold NLRP1 interacting via PYD with the adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC, also known as PYCARD) which can then recruit the inflammatory procaspase, caspase-1 (also known as IL-1β-converting enzyme or ICE). Upon sensing of putative ligands and subsequent inflammasome activation, procaspase-1 is autocatalytically cleaved and activated (Wilson et al., 1994). Active caspase-1 can then process IL-1β and IL-18 and the biologically active cytokines are secreted in an unconventional Golgi/endoplasmic reticulum-independent manner (Keller et al., 2008). The human NLRP1 inflammasome was shown to interact with the bacterial peptidoglycan MDP in a cell-free system (Faustin et al., 2007; Hsu et al., 2008) and can be activated in keratinocytes by ultraviolet B (UVB) irradiation (Feldmeyer et al., 2007). The mechanisms involved in its activation remain unclear. Mutations in the *NLRP1* gene have been linked to susceptibility to vitiligo-associated autoimmune diseases (Jin et al., 2007), systemic lupus erythematosus and rheumatoid arthritis (RA) (Masters, 2013). Gain-of-function mutations of the *NLRP1* gene were recently described in two skin disorders, namely multiple self-healing palmoplantar carcinoma and familial keratosis lichenoides chronica. These *NLRP1* mutations result in the blockade of the autoinhibitory effect of NLRP1 LRR and PYD domains and lead to an increased activation of the inflammasome with C-terminal CARD-ASC binding (Zhong et al., 2016). Recently, it has been demonstrated that anthrax lethal factor can cleave the PYD domain of murine NLRP1, but not human NLRP1, causing its activation. This observation identifies proteolytic cleavage as a NRLP1 activation

mechanism for the murine NLRP1 (Chavarria-Smith et al., 2016). Moreover, these studies showed that NLRP1 PYD domain is dispensable for inflammasome activity but that ASC is required via NLRP1 CARD domain interaction, contrarily to the first observations (Martinon et al., 2002; Zhong et al., 2016).

The **NLRP3 inflammasome** is the best characterized inflammasome to date, and a broad range of stimuli can induce its activation. These include PAMPs such as lipopolysaccharide (LPS), fungal zymosan, bacterial toxins, and also the bacteria *Listeria monocytogenes* (Meixenberger et al., 2010), *Staphylococcus aureus* (Munoz-Planillo et al., 2009), and *Propionibacterium acnes* (Kistowska et al., 2014b; Qin et al., 2014), as well as yeasts like *Candida albicans* (Hise et al., 2009) and of the *Malassezia* spp. (Kistowska et al., 2014a). NLRP3 can also be activated by danger-associated molecules that are not derived from pathogens but often associated with cellular stress, the so-called DAMPs, including extracellular ATP (Mariathasan et al., 2006), asbestos (Dostert et al., 2008), amyloid-β (Halle et al., 2008), DNA:RNA hybrids (Kailasan Vanaja et al., 2014), and crystals such as gout-causing monosodium urate (MSU) (Martinon et al., 2006), silica (Dostert et al., 2008), or cholesterol (Duewell et al., 2010). Interestingly, the study of patients with autosomal dominant cold-induced urticaria, later termed familial cold autoinflammatory syndrome (FCAS), allowed the identification of mutations in the *CIAS1*/cryopyrin/*NLRP3* gene (Hoffman et al., 2001). These studies permitted major advances in the identification and understanding of autoinflammatory diseases but also resulted in a gain of interest in IL-1β biology and its role in inflammatory disorders.

Since such a broad range of stimuli can activate the NLRP3 inflammasome, it is believed that a common mechanism triggered by diverse activators leads to NLRP3 activation. Several events such as the release of oxidized mitochondrial DNA (Shimada et al., 2012), production of reactive oxygen species (ROS) (Dostert et al., 2008), mitochondrial stress (Zhou et al., 2011), lysosomal rupture with cathepsin B release (Hornung et al., 2008), changes in intracellular calcium (Ca^{2+}) levels (Murakami et al., 2012) and potassium (K^+)-efflux (Petrilli et al., 2007) have been reported to be associated to inflammasome activation (Figure 4). Whether all or only a part of these events are required for NLRP3 inflammasome activation is not clear. In 2013, Muñoz-Planillo R *et al*. suggested that the sole reduction of intracellular K^+ was sufficient for NLRP3 inflammasome activation (Munoz-Planillo et al., 2013) but recent reports have suggested that, in certain circumstances, inflammasome activation can occur independently of K^+-efflux (Gross et al., 2016) or phagocytosis of bacteria (Chen et al., 2016). ROS were shown to activate NEK7, a kinase involved in the control of mitosis, causing its direct binding to the LRR domain of NLRP3 and modulating its function (He et al., 2016; Shi et al., 2016). Moreover, NLRP3 oligomerization has been shown to be hindered by ubiquitination (Juliana et al., 2012; Py et al., 2013), nitrosylation (Hernandez-Cuellar et al., 2012; Mao et al., 2013; Mishra et al., 2013) and phosphorylation on its LRR and PYD domains (Spalinger et al., 2016; Stutz et al., 2017). The activity of the NRLP3 inflammasome has also been reported to be under the control of several kinases (discussed in chapter

1.3). Currently, a consensual and unifying mechanism leading to NLRP3 inflammasome remains a matter of intense debate and investigation.

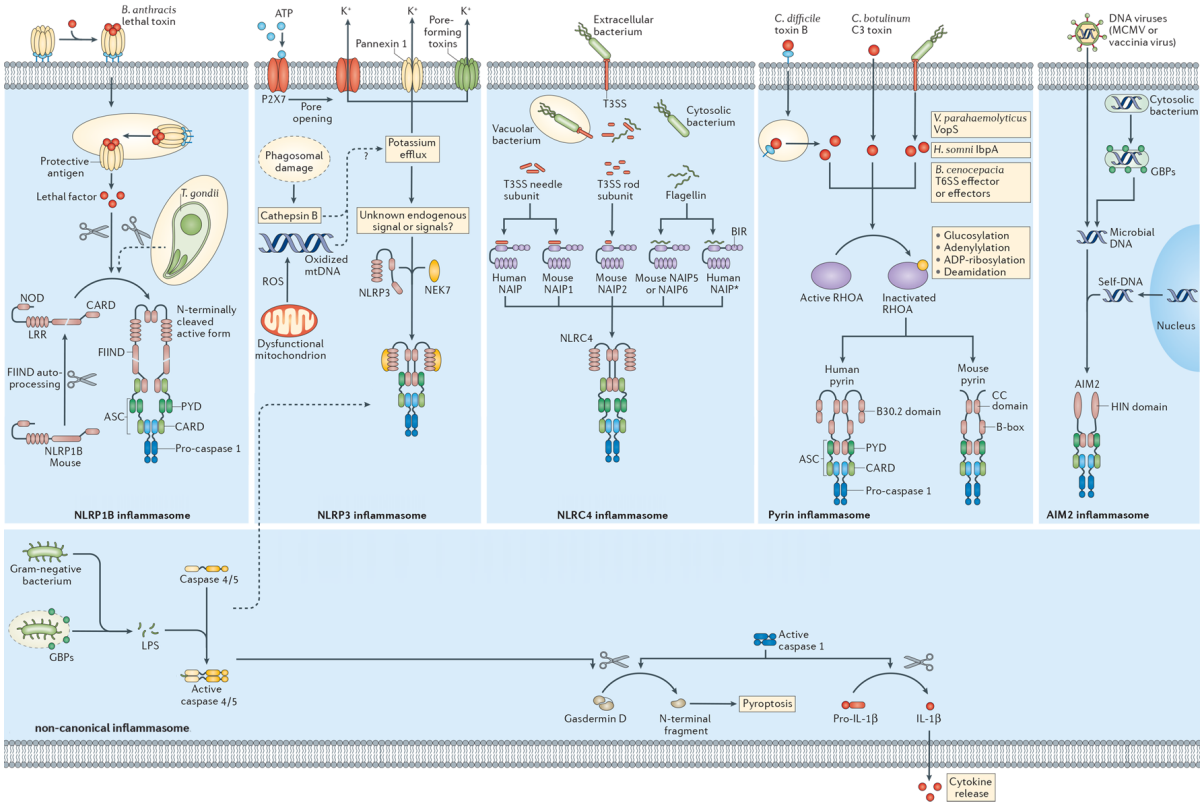

Figure 4 The activation pathway of the main inflammasome complexes

Mouse NLRP1 responds to *Bacillus anthracis* lethal factor and *Toxoplasma gondii* infection. Several events such as extracellular ATP-mediated potassium (K^+) efflux, phagosomal damage with cathepsin B release, reactive oxygen species (ROS) or release of oxidized mitochondrial DNA (mtDNA), drive NEK7 interaction with NLRP3. NLR family, apoptosis inhibitory proteins (NAIPs) sense bacterial flagellin and type 3 secretion system (T3SS) with further recruitment and activation of NLRC4. Pyrin activation depends on detection of inactivated RhoA by bacterial toxins or effector protein. AIM2 binds to double-stranded host or microbial DNA in the cytosol. Cytosolic LPS directly binds caspase-4 or caspase-5, which activate gasdermin D and NLRP3 resulting in pyroptotic cell death. For details refer to the text. Adapted from (Broz and Dixit, 2016).

The absent in melanoma 2 or **AIM2 inflammasome** recognizes self, viral and bacterial double-stranded DNA (dsDNA) via its HIN-200 domain (Muruve et al., 2008). AIM2, like NLRP3, recruits caspase-1 via the adaptor protein ASC. Increased levels of AIM2 were found in keratinocytes of patients with psoriasis and atopic dermatitis, causing acute and chronic skin barrier disruption-related inflammation (Ito et al., 2015).

The **NLRC4 inflammasome** is activated by bacterial flagellin (Mariathasan et al., 2004) and type 3 secretion system (T3SS) proteins (Miao et al., 2010). NLRC4 contains a CARD domain and is therefore able to recruit and activate caspase-1 without the adaptor ASC. Interestingly, efficient cytokine production has been described to require ASC (Chen et al., 2014; Denes et al., 2015), whereas the direct recruitment of caspase-1 results in cell death (Broz et al., 2010). *Salmonella typhimurium* flagellin and T3SS needle subunit of *Chromobacterium violaceum* are recognized in human macrophages by the

NLR family, apoptosis inhibitory protein (NAIP) which interacts with NLRC4, thus triggering its assembly (Zhao et al., 2011; Kortmann et al., 2015). *Salmonella* has also been reported to activate NLRC4 by inducing phosphorylation at its serine 533 residue by protein kinase C δ-type (PKCδ) (Qu et al., 2012). Moreover, NLRC4 can recruit NLRP3 resulting in increased caspase-1 processing (Qu et al., 2016). Reported *NLRC4* gene mutations cause recurrent fever flares and macrophage activation syndrome (MAS) (Canna et al., 2014), neonatal-onset enterocolitis and fatal or near-fatal episodes of autoinflammation (Romberg et al., 2014).

The **pyrin inflammasome** is encoded by the *MEFV* gene and contains PYD, TRIM and B30.2 domains. This inflammasome is activated by bacterial toxins like *Clostridium difficile* toxin B (TcdB) and C3 toxins which modify the GTPase RhoA impairing the activation of the serine-threonine protein kinase N1 (PKN1) and PKN2. In normal conditions, these kinases can bind and phosphorylate pyrin on its 14-3-3 interaction motif (Park et al., 2016). Interaction of phosphorylated pyrin and the scaffolding proteins 14-3-3τ and 14-3-3ϵ has been associated with nuclear compartmentalization which impairs inflammasome formation (Jeru et al., 2005). Pyrin is involved in autophagy and its B30.2 domain is involved in the recognition of target molecules, such as NLRP1, NLRP3 and pro-caspase-1 (Kimura et al., 2015). Mutations in this gene are the cause of familial Mediterranean fever (FMF) (Chae et al., 2006) and the recently described disease-entity pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND) (Masters et al., 2016). Mevalonate kinase deficiency (MKD) was also linked to the activation of the pyrin inflammasome: a defect in RhoA geranylgeranylation causes its inactivation impairing PNKs activity (Park et al., 2016).

Shorter proteins containing only pyrin or CARD domains, called PYD-only proteins (**POPs**) and CARD-only proteins (**COPs**), respectively, can disrupt inflammasome assembly by blocking the oligomerization process (Dorfleutner et al., 2015). POP1 and POP3 were shown to interact with ASC and thus competing in binding to NLRP3 or AIM2, respectively, regulating and quenching their activities (Khare et al., 2014; de Almeida et al., 2015). POP2, instead, interacts directly with the PYD-domain of NLRP3 but it can also inhibit NF-κB transactivation (Atianand and Harton, 2011; Ratsimandresey et al., 2017) in a similar way to POP4 (NLRP2P), which in turn doesn't affect inflammasome assembly (Porter et al., 2014). Some viruses, such as the myxoma poxvirus and the Shope fibroma virus, express viral POPs (vPOPs) to evade the immune system (Johnston et al., 2005; Dorfleutner et al., 2007). The COPs INCA (CARD17) and ICEBERG (CARD18) block caspase-1 activation by interacting with its CARD domain (Lu et al., 2016). CARD18 is expressed mainly by differentiated keratinocytes and in response to UVB (Qin et al., 2017) and it is increased in psoriatic epidermis (Goblos et al., 2016).

Other less characterized inflammasomes include NLRC1 (NOD1) and NLRC2 (NOD2), both previously discussed, NLRC3 and NLRP12. Besides their role in caspase-1 activation, they modulate NF-κB as well as MAPK pathways and autophagy (Lupfer and Kanneganti, 2013). NRLP12 was linked to inflammasome formation upon recognition of acylated lipid A from *Yersinia pestis* infection

(Vladimer et al., 2012) but it is also able to negatively modulate the activity of the NF-κB by interacting with its mediators IRAK1, TRAF3 and NF-κB-inducing kinase (NIK) (Williams et al., 2005; Allen et al., 2012). Similarly, NLRC3 exerts its inhibitory functions by interacting with TRAF6 (Schneider et al., 2012).

The involvement of caspase-5 in the processing of IL-1 was already described in the original publication describing the inflammasome (Martinon et al., 2002), but it is only a decade later that its role in cell death was reported (Kayagaki et al., 2011; Kayagaki et al., 2015). Human caspase-4, caspase-5 and the orthologous murine caspase-11 are activated by cytosolic LPS or by the release of pathogens from vacuoles mediated by interferon-inducible GTPase guanylate-binding proteins (GBPs) (Pilla et al., 2014; Shi et al., 2014). Then, the activated caspases cleave the substrate gasdermin D (GSDMD) creating pores in the cell membrane and resulting in pyroptosis, a form of inflammatory programmed cell death distinct from apoptosis (He et al., 2015; Shi et al., 2015). Processing of GSDMD was also linked to the formation of the NLRP3 inflammasome and subsequent IL-1β and IL-18 secretion in a process known as the **non-canonical inflammasome** activation (Man and Kanneganti, 2016). The gasdermin family comprises 4 paralogous genes in humans. Very little is known about the other three isoforms, which, in contrast to GSDMD, do not possess a caspase cleavage site. Gasdermin D has N-terminal domain that can bind lipid membranes and form pores (Ding et al., 2016) and a C-terminal domain that inhibits pyroptotic functions but can be cleaved by caspases (Aglietti and Dueber, 2017).

Pyroptosis is characterized by cellular and organelle swelling driving plasma membrane rupture with release of intracellular pathogens but also strong immunogenic cellular contents including the DAMPs high mobility group box-1 (HMGB1), ATP and uric acid (Fink and Cookson, 2005). Pyroptosis plays therefore a key role in responses to cytosolic pathogens, eliminating their protective replication niche and allowing other immune cells to eliminate them (Jorgensen and Miao, 2015). Interestingly, GSDMD was found to be necessary for caspase-11-driven pyroptosis but *Gsdmd*$^{-/-}$ mice were not completely protected from inflammatory cell death (Kayagaki et al., 2015). This suggests the existence of other pyroptotic mediators that may be substrates of other inflammatory caspases, namely caspase-1 and caspase-8. Indeed, caspase-1 has been considered as the principal driver of pyroptosis (Bergsbaken et al., 2009). Interestingly, caspase-1 is also involved in apoptotic cell death in keratinocytes irradiated with UVB (Sollberger et al., 2015). Apoptosis is the most prominent and well-studied form of programmed cells death: activation of the executioner caspase-3 and caspase-7 results in cell shrinkage, chromatin condensation, DNA fragmentation and membrane blebbing (Samali et al., 1999). The cells break apart into non-immunogenic vesicles, termed apoptotic bodies, which are then target for phagocytosis (Erwig and Henson, 2008). Activation of this process is regulated by strict mechanisms: in the intrinsic pathway, intracellular signal cause Bcl-2 family members' migration and destabilization of mitochondria resulting in the release of cytochrome c, formation of the apoptosome and caspase-9 activation (Czabotar et al., 2014). The extrinsic pathway activation involves extracellular death ligands like TNFα or Fas ligand which cause the formation of the death-inducing signaling complex (DISC)

and activation of caspase-8 (Elmore, 2007). Initiator caspase-8 and caspase-9 then activate the executioner caspase-3 and caspase-7 resulting in cell death.

Processing of IL-1β can occur either in a caspase-1/inflammasome-dependent or independent manner. Indeed, caspase-8, an initiator caspase mainly involved in apoptosis, can be involved in the activation of the NF-κB pathway and IL-1β/IL-18 processing. Recognition by dectin-1 of extracellular fungi such as *Candida albicans* results in the formation of a complex with CARD9, Bcl-10, MALT1, ASC and caspase-8 which, once activated, can directly process IL-1β. In contrast, the intracellular recognition of fungi by dectin-1 drives the NLRP3 inflammasome (Gringhuis et al., 2012). *Salmonella* infection has also been reported to activate both caspase-1 and caspase-8 in a NLRC4-ASC-dependent manner (Man et al., 2013). Moreover, activation of caspase-8 through the Fas-signaling pathway can also lead to the direct processing of IL-1β and IL-18 independently of caspase-1 and ASC (Bossaller et al., 2012). Other enzymes known to lead to the processing of IL-1 family members include neutrophil-derived elastase, cathepsin G and proteinase 3 (myeloblastin), mast cell-derived chymase and granzyme B from cytotoxic lymphocytes and NK cells. Extracellular proteases play a fundamental role in processing the pro-form of cytokines released by pyroptotic cells, therefore complementing caspase-1 in their activation (Afonina et al., 2015).

INTERLEUKIN-1 FAMILY

Cytokines comprise a variety of molecules secreted by immune and non-immune cells that regulate important cellular functions and physiological processes especially in the hematopoietic and immune systems. One important class of cytokines are the interleukins, a large family of small secreted proteins that bind to specific membrane receptors on target cells. The history of interleukins, and particularly of interleukin-1 (IL-1), began in 1948 when Paul B. Beeson discovered an active unknown substance obtained from rabbit leukocytes that was able to cause fever (Beeson, 1948). Decades later, Charles Dinarello identified two chemically and biologically distinct pyrogenic molecules produced by neutrophils and monocytes incubated with heat-killed *Staphylococcus albus*; he named them human leukocytic pyrogens (LP) (Dinarello et al., 1974; Dinarello et al., 1977). Before him, Igal Gery reported that the stimulation of murine and human lymphocytes with lipopolysaccharide (LPS), an essential component of gram-negative bacteria, led to the release of a soluble factor that was able to enhance the response of T lymphocytes to lectins (phytohemagglutinin and concanavalin A) (Gery et al., 1972). In 1979, the molecules with inflammatory properties reported by Charles Dinarello and Igal Gery revealed to be the same, namely IL-1 (Rosenwasser et al., 1979). Following progress in sequencing technologies, it turned out that the IL-1 family comprises a total of eleven members with similar or distinct biological effects. In addition to IL-1α and β, IL-18, IL-33, IL-36α, β and γ are proinflammatory, whilst, antagonists to IL-1 and IL-36 receptors (IL-1Ra, IL-36Ra), IL-37 and IL-38 are anti-inflammatory. Genes encoding IL-1 family members are mostly located on human chromosome 2 with two exceptions,

namely the genes encoding IL-18 and IL-33 that are located on chromosomes 11 and 9, respectively. IL-1 cytokines bind to and act through specific receptors, which are characterized by an intracellular TIR domain (shared with TLRs) and an extracellular immunoglobulin-like binding domain (Boraschi and Tagliabue, 2013). The IL-1 receptor family comprises several members including IL-1R1, the decoy receptor IL-1R2, IL-1R accessory protein (IL-1RaP or IL-1R3), IL-1R4 (T1 or ST2), IL-18Rα (IL-1R5), IL-36R (IL-1R6), IL-18R accessory protein (IL-18Rβ or IL-1R7), IL-1R8 (TIR8 or SIGIRR), IL-1R9 (IL-1RAPL2 or TIGIRR-1) and IL-1R10 (TIGIRR-2) (Figure 5). IL-1α/IL-1β, IL-18 and IL-36 initiate immune and inflammatory responses by binding to IL-1R1, IL-18Rα and IL-36R, respectively.

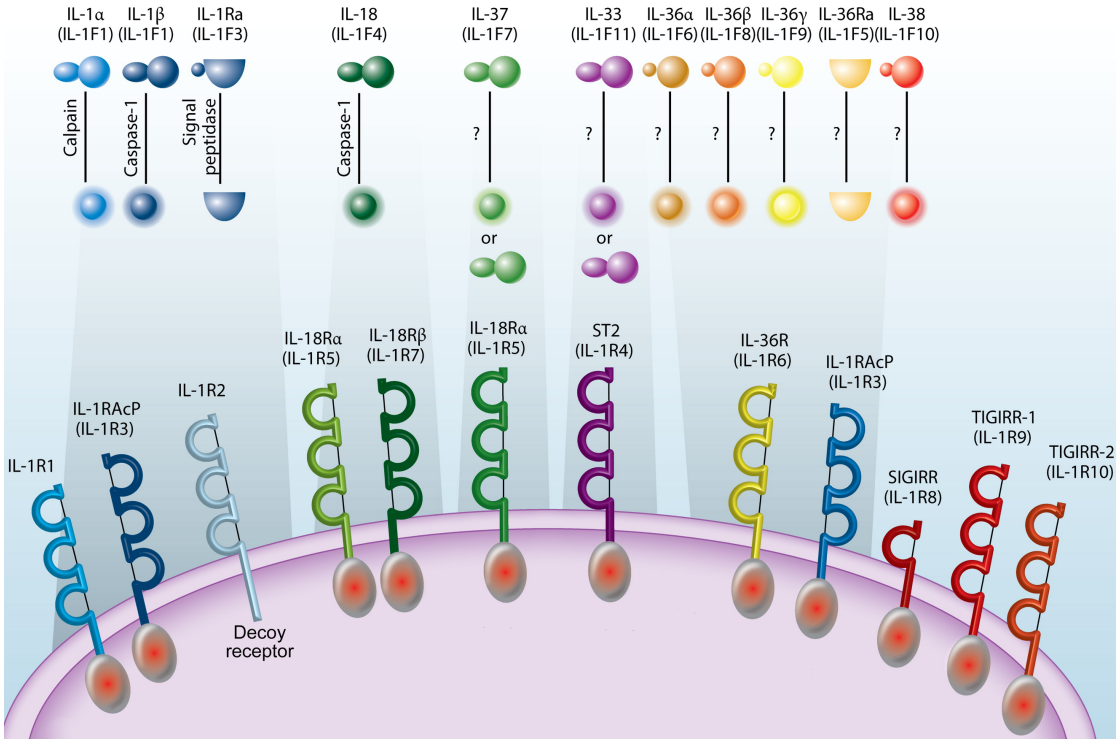

Figure 5 IL-1 cytokines and their receptors

Cytokines and receptors of the IL-1 family. Mature IL-1α, IL-1β and IL-1Ra bind to the IL-1 receptor formed by IL-1R1 and IL-1RAcP; IL-1R2 lacks the cytosolic TIR domain and functions a decoy receptor. Mature IL-18 binds to IL-18Rα:IL18-Rβ. Mature IL-36α/β/γ bind to IL-36R and IL-1RAcP. Adapted from (Netea et al., 2015).

The co-receptor IL-1RaP interacts with IL-1R1, IL-1R2, IL-1R4 and IL-36R while IL-18Rβ is a unique accessory chain for IL-18Rα. The decoy receptor IL-1R2 lacks the cytoplasmic TIR domains and is therefore unable to initiate a signaling cascade even in the presence of its accessory receptor. IL-1R2 binds IL-1β with high affinity and IL-1α or IL-1Ra with low affinity (Symons et al., 1995). The biological activity of IL-1 family cytokines is tightly regulated not only by decoy receptors but also by soluble receptor antagonists such as IL-1Ra and IL-36Ra that can specifically antagonize IL-1α, IL-1β and IL-36. In addition, IL-1R1 can be released into the extracellular space where, in its soluble form (sIL-1R1), can also function as a soluble decoy receptor and prevent the binding of IL-1α, IL-1β and IL-1Ra to membrane IL-1R1 (Burger et al., 1995). Furthermore, IL-1R2 can also be cleaved and

solubilized by metalloproteinases resulting in an increased segregation of IL-1β due to its higher affinity (Symons et al., 1995).

INTERLEUKIN-1 AND INTERLEUKIN-18

IL-1β acts on a broad range of cell types and is a key mediator of the acute phase of inflammation inducing local and systemic responses (Dinarello, 2009; 2011). IL-1R-mediated signaling resembles TLR signaling: in fact, the cytosolic TIR domains of IL-1R1 and IL-1RAcP recruit the adaptor MyD88 which in turn activates the IRAK1/2/4-TRAF6 complex. Following recruitment and ubiquitination of the MAP3K TGF-β-activated kinase 1 (TAK1) a signaling cascade trigger p38 and JNK mitogen-associated protein kinases (MAPKs) and the transcription factors NF-κB (Dai et al., 2012). IL-1β effects are numerous and include the secretion of downstream proinflammatory mediators such as cyclooxygenase type-2 (COX-2), IL-6, TNF and IL-1 itself (Dinarello, 1996; Weber et al., 2010). In the body, the inflammatory effects of IL-1 manifest as fever, vasodilation and hypotension as well as an increased sensitivity to pain. The pyrogenic activity of IL-1 is due to the activation of NF-κB and the resulting expression of COX-2, an enzyme involved in the synthesis of prostaglandins (Lee et al., 2004). Moreover, IL-1 plays an important role in leukocyte trafficking and activation, T cell and B cell differentiation with production of immunoglobulin G (IgG), IgM and IgA (Akdis et al., 2016). IL-1β is not only secreted by immune cells such as monocytes/macrophages, dendritic cells, neutrophils, B lymphocytes and NK cells but also by non-immune cells such as keratinocytes (Dinarello, 2009; Feldmeyer et al., 2010).

First identified and described as interferon-γ-inducing factor (IGIF) (Nakamura et al., 1989; Nakamura et al., 1993), IL-18 received its current name three years later (Ushio et al., 1996). In contrast to the strong pyrogenic activity of IL-1α and IL-1β, IL-18 is only able to induce fever at higher concentrations. IL-18 activates primarily p38 MAPK and AP-1, but fails to activate NF-κB (Lee et al., 2004). IL-18 activity is mainly regulated by a soluble protein called IL-18 binding protein (IL-18BP). IL-18BP differs from the other soluble IL-1 receptors because it retains a unique binding sequence composed by a single immunoglobulin domain (Dinarello et al., 2013). Similar to the IL-1Rs, IL-18Rs can also be found in a soluble form that is used as a biomarker for inflammatory diseases such as RA and adult-onset Still's disease (Takei et al., 2011). IL-18 activates NK cells, synergizes with IL-12 to drives T_H1 response and IFNγ production (Tominaga et al., 2000; Akdis et al., 2016).

Both IL-1β and IL-18 are first synthesized as precursors, which need to be processed into their biologically active form by an inflammasome. In contrast, both pro and cleaved forms of IL-1α are biologically active and induce, via IL-1R1 signaling, the production of TNFα and IL-6 in human A549 epithelial cells and peripheral blood mononuclear cells (PBMCs) (Kim et al., 2013). The transcription of the IL-1α gene is regulated by a variety of stimuli including proinflammatory or stress-associated stimuli and growth factors (Di Paolo and Shayakhmetov, 2016). Pro-IL-1α lacks a signal secretion

peptide. However, its release from dying cells is able to trigger acute inflammation (Chen et al., 2007). IL-1α can be translocated to the plasma membrane where it signals in an intra and paracrine manner but can also be secreted in its mature form via both IL-1β-dependent or independent pathways (Fettelschoss et al., 2011; Gross et al., 2012). Several inflammatory proteases, including calpain, elastase, granzyme B, and mast cell chymase, can cleave IL-1α converting the cytokine into its more potent form (Afonina et al., 2011). Since pro-IL-1α contains a nuclear localization signal, it can induce the expression of proinflammatory genes independently of IL-1R1 signaling (Werman et al., 2004). Because of the multiplicity of mechanisms of action of IL-1α, it plays an important role in the maintenance of homeostasis and the pathology of several human diseases (Di Paolo and Shayakhmetov, 2016).

1.2 THE SKIN

The skin is one of the largest organs of the body with an total area[a] of 2 m^2 and a weights of 5 kg (McGrath and Uitto, 2016). It forms a barrier that protects the host from environmental insults of various origin, such as chemicals and irritants, ultraviolet (UV) radiation and pathogens. Chemicals fail to penetrate the skin because of its stratified organization, which also prevents water loss from the organism. The skin is formed by two structures: the epidermis, composed mainly of keratinocytes, and the dermis, a connective tissue made of extracellular-matrix produced by fibroblasts and containing supportive systems like blood and lymphatic vessels (Figure 6). Dermis and epidermis are connected and anchored together by the basement membrane. Several immune cells, such as tissue resident Langerhans cells and T cells, macrophages, dermal DCs and ILCs resides in the skin.

[a] The standard skin surface of 2 m^2 is obtained considering the skin as a flat plane and its therefore a good reference for calculations regarding water loss, exposure to UV light or topical application of non-penetrating drugs. But the skin includes several appendages such hair follicles and sweat ducts which greatly increase the epithelial surface accessible to the microbiome. Richard Gallo recalculated the skin surface by including those appendages and obtained a surface area of approximated 24 m^2 (Gallo, 2017).

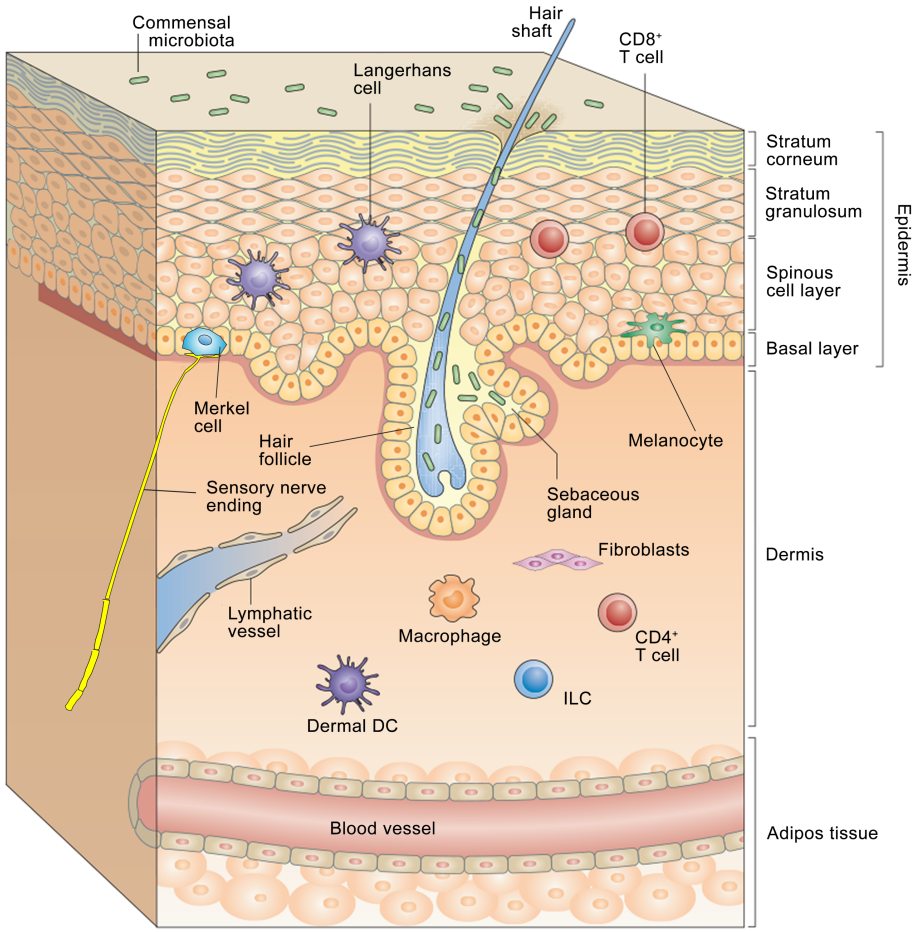

Figure 6 The structure of the human skin

Representation of the human skin with main structures and cells found in the epidermis and dermis. The upper part (epidermis) is formed by stratified keratinocytes: stratum basale, spinosum, granulosum, lucidum (not shown) and corneum. The dermis is mainly composed by extracellular matrix produced by fibroblast and contains several structures such as sebaceous glands, hair follicles, blood and lymphatic vessels. Adapted from (Pasparakis et al., 2014).

THE DERMIS

The dermis is a layer of the skin located between the subcutaneous tissues (hypodermis) and the upper and outermost epidermis. The main component of the dermis is the extracellular matrix, a collagen-rich milieu produced by fibroblasts. In comparison to the epidermis, the dermis is less stratified but contains several structures like hair follicles with sebaceous glands, blood and lymphatic vessels, sweat glands and terminal nerves (ending of nerve cells) (McGrath and Uitto, 2016). The hair follicles, the sebaceous and sweat glands are considered epidermal appendages since they develop from the epidermis into the dermis as downgrowths or diverticula. They remain separated from the dermis by the basement membrane. Eccrine sweets gland and blood vessels play an important role in the thermoregulation by releasing sweat and vasodilation to cool the body or by vasoconstriction to reduce heat loss (Charkoudian, 2010; Noel et al., 2012). Other than fibroblasts, vessel-forming epithelial cells, macrophages, dermal DCs, $CD4^+$ T cells and ILCs are found the dermis. At the steady state, the dermis is populated by three subsets of dermal DCs (DDCs) characterized by the expression of CD1c, CD141

and CD14 (Malissen et al., 2014). They are able to activate and induce proliferation of dermal $CD4^+$ T but also to migrate to skin-draining lymph nodes where they preferentially induce T_H2 polarization (Boltjes and van Wijk, 2014). Moreover, $CD141^+$ DDCs excel in exogenous antigen cross-presentation and are therefore important in the recognition of tumor- or viral infected-derived antigens and activation of cytotoxic T cells (Tullett et al., 2014). ILCs are essential innate cells involved in skin homeostasis but also involved in the pathogenesis of detrimental inflammatory disease such as psoriasis and atopic dermatitis (Kim, 2015; Klose and Artis, 2016).

THE EPIDERMIS

The epidermis is the outermost layer of the skin and serves as the first line of defense against physical, microbial and chemical insults. It is formed by stratified keratinocytes proliferating from the lower basal layer (stratum basale or stratum germinativum) to the differentiated spinous layer (stratum spinosum), granular layer (stratum granulosum) and the upper stratum corneum. The thick skin of the palms and soles (palmoplantar skin) has an additional layer between the strati granulosum and corneum termed stratum lucidum (McGrath and Uitto, 2016). Keratinocytes account for 95% of epidermal cells. Other cells composing the epidermis are melanocytes, $CD8^+$ T cells, Langerhans cells and Merkel cells. Melanocytes are specialized cell in the production of melanin, the dark pigment responsible for the skin color[a], which is also found in hairs and in the tissue underlying the iris. In the skin, melanocytes reside in the bottom layer of the epidermis, the basal layer, and in hair follicles (Cichorek et al., 2013). Packages of melanin (melanosomes) are then distributed by melanocyte dendrites to the adjacent keratinocytes and accumulate in the perinuclear area. Melanin plays an import role in the protection of DNA damage by absorbing the energy from UV radiation (Brenner and Hearing, 2008). Merkel cells are tactile cells responsible for light touch sensation. They are also located in the basal layer of the epidermis where they form synaptic contacts with the ending of sensory nerve cells extruding from to the dermis (Maksimovic et al., 2014). Langerhans cells (LCs) are a radio-resistant subset of dendritic cells (Merad et al., 2008) accounting for the 3-5% of epidermal cells and are the only professional APCs found in homeostatic conditions. They act as sentinels for foreign antigens (Kashem et al., 2017). They continuously migrate to local skin-draining lymph nodes but by encountering of antigens they increase their migration rate in order to initiate an effective adaptive immune response in the skin (Stoitzner et al., 2005) Moreover, they are also able to cross-present exogenous antigens to $CD8^+$ T cells (Stoitzner et al., 2006). Proliferation of LCs in very limited, just allowing to preserve a constant number of cells in the epidermis; their regeneration is supported by keratinocytes IL-34 at normal condition and by macrophage colony-stimulating factor (M-CSF) from neutrophils during inflammation (Wang et al.,

[a] Skin color is determined by the amount and nature of melanin present in the skin but is not correlated with the quantity of melanocytes. In fact, dark skin people have similar distribution of melanocytes as lighter human races. The average distribution is about 1200 melanocytes per mm^2 of skin (Cichorek et al., 2013).

2016b). Tissue-resident memory T cells are also found and retained in the epidermis: they predominate at sites of previous infections or inflammation and therefore facilitate immune surveillance against reinfections (Gebhardt et al., 2009; Mueller et al., 2014). Keratinocytes are also active players in the immunological response to pathogens. In addition to their barrier functions, they are an important source of AMPs such as β-defensins and cathelicidins. Keratinocytes express all TLRs except TLR7[a] and TLR8 (Lebre et al., 2007) and they can therefore sense PAMPs. They constitutively express both pro-IL-1α and pro-IL-1β (Nestle et al., 2009) and they have functional inflammasomes (discussed below in details) (Feldmeyer et al., 2007). Moreover, keratinocytes express MHC-II upon stimulation with IFNγ and can act as non-professional APCs (Takagi et al., 2006); in fact, it was reported that they possibly activate both $CD4^+$ and $CD8^+$ T cells (Fan et al., 2003; Kim et al., 2009).

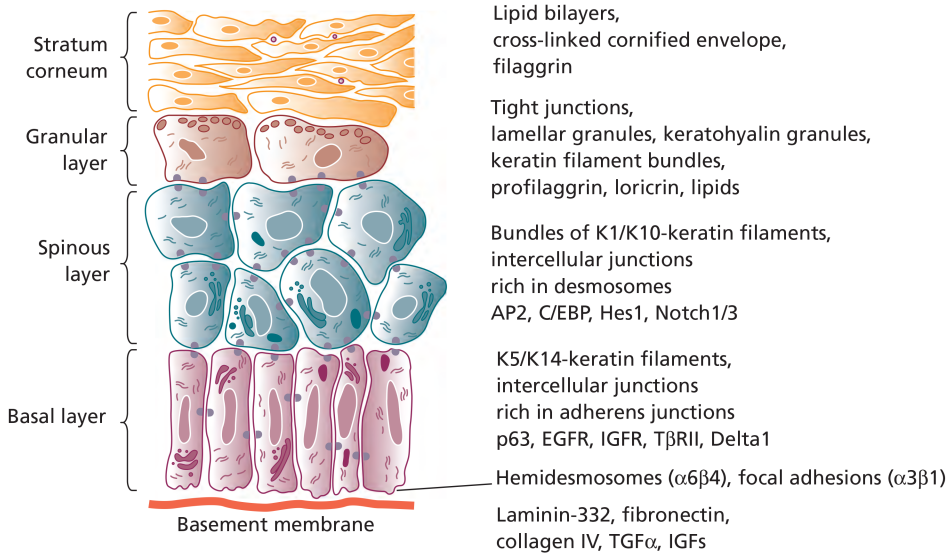

Figure 7 The process of epidermal differentiation

Proliferative keratinocytes differentiate from the basal layers to the stratum corneum. During this process, they express different structural protein (keratins, adherens junctions, desmosomes, tight junctions) regulated by specific transcription factors and signaling molecules. Figure from (McGrath and Uitto, 2016).

The differentiation of the keratinocytes is a sequential process that includes the expression of different cellular structural and junctional elements and requires 30 days for cellular progression from stratum basale to stratum corneum (McGrath and Uitto, 2016). The basal layer is composed by a continuous layer of small cuboidal cells anchored to the basement membrane by hemidesmosomes (Walko et al., 2015). Keratinocytes cell-cell adhesion is secured by several structures like desmosomes, adherens junctions and tight junctions (Figure 7). Desmosomes constitute the major adherent structure that anchors keratinocytes: they connect cytosolic keratin filaments to adjacent cells via transmembranous cadherins (desmogleins and desmocollins) (Kowalczyk and Green, 2013). Adherens junctions bridge

[a] TLR7 expression can be induced by dsRNA triggering of TLR3 (Kalali et al., 2008).

cells via cadherin or nectin transmembrane proteins and form complexes with the actin cytoskeleton, whereas tight junctions seal adjacent cells together via claudins, occludins or junctional adhesion molecule interaction (Niessen, 2007). Tight junctions create a solid barrier and so they regulate permeability and diffusion of ions and solutes (Zihni et al., 2016). Proliferating cells from the basal layer start to differentiate by overexpressing desmosomes and switching their keratin expression from keratin 5/keratin 14 (K5/K14) to K1/K10. Keratins are a key component of intermediate filaments and, together with filaggrin, they constitute 80-90% of the epidermis mass (McGrath and Uitto, 2016). There are 54 functional *keratin* genes, each of which has a specific expression pattern (Pan et al., 2013). The interaction between keratins and desmosomes cause the shrinking of the microfilaments resulting in the prickle cell shapes typical of the stratum spinosum. Cells in the granular layer are interconnected by tight junctions. They produce lamellar bodies and keratohyalin granules containing profilaggrin and loricrin, proteins rich in cysteine residues that bind together the keratin bundles of the last stratum of the epidermis. The stratum corneum is formed by 15-20 layers of terminally differentiated dead keratinocytes which have lost the nuclei and cytoplasmic organelles called corneocytes. They are formed mainly of involucrin and loricrin but also contain several lipids released by lamellar bodies which create an impermeable sheath. These granules also release proteases important for desquamation (skin peeling), the process that allows the epidermis to continuously renew (Menon et al., 1992).

IL-1, INFLAMMASOMES AND SKIN DISEASES

Keratinocytes represent the great majority of epidermal cells and, in addition to their role in the barrier function of the skin, are able to exert critical functions in innate immunity. Actually, and although inflammasomes have been mainly characterized in myeloid immune cells, inflammasomes are also expressed and functional in human keratinocytes (Beer et al., 2014). Indeed, UVB irradiation activates the NLRP1 and NLRP3 inflammasomes in keratinocytes with subsequent IL-1β release (Feldmeyer et al., 2007). Reddening of the skin, swelling, pain and fever are all symptoms of sunburn related to strong inflammatory properties of IL-1β. UVB irradiation also induces apoptotic cell death in keratinocytes and this process depends on caspase-1 activity (Sollberger et al., 2015). The NLRP3 inflammasome in keratinocytes is able to sense different contact sensitizers and is involved in the sensitization phase of contact hypersensitivity (CHS) (Watanabe et al., 2007). Also, it has been reported that inflammasomes are involved in skin homeostasis and inflammatory skin diseases (Contassot et al., 2012; Satoh et al., 2015). Acne vulgaris is a common inflammatory and potentially severe skin disease associated with colonization of the pilo-sebaceous unit by the commensal bacterium *Propionibacterium acnes*. *P. acnes* is considered to contribute to inflammation in acne and has been shown to activate the NLRP3 inflammasome in human monocytes (Kistowska et al., 2014b; Qin et al., 2014) and in sebocytes (Li et al., 2014). Therefore, IL-1β is thought to play an important role in acne pathogenesis. Studies involving the blockage of IL-α and IL-β with neutralizing antibodies have shown a clinical response associated

with reduction of inflammatory acne lesions (XOMA, 2013; Carrasco et al., 2015). The fungal genus *Malassezia* is linked to several inflammatory skin diseases such as seborrheic dermatitis (seborrheic eczema), pityriasis versicolor (tinea versicolor), atopic eczema, psoriasis, *Malassezia* folliculitis and Onychomycoses (Gaitanis et al., 2012). The etiological agent of pityriasis versicolor, *Malassezia* was shown to activate the NLRP3 inflammasome via the dectin-1 and Syk signaling cascade, causing the release of IL-1β (Kistowska et al., 2014a). Another common disorder that affects primarily the skin and the joints is psoriasis, a chronic inflammatory immune-mediated inflammatory disease affecting 1.5-2% of the Western industrialized countries (Johansen et al., 2005). Psoriasis vulgaris manifests as red, scaly patches of the skin. In lesions, keratinocytes express IL-1α, IL-1β and IL-18 which regulate the expression of genes involved in the pathogenesis of psoriasis including the antimicrobial peptides S100A7 and LL-37 (Perera et al., 2012). Also, UV irradiated keratinocytes express granzyme B (Hernandez-Pigeon et al., 2006) and its release may contribute to skin inflammation by processing and activation of IL-18 in a non-cytotoxic manner (Afonina et al., 2015). Inflammation in the skin with extensive release of IL-1β is often associated with neutrophilic infiltration as first line of defense. In the absence of infection, neutrophils can become detrimental for the host by causing tissue damage (Navarini et al., 2016). Inflammatory skin diseases are, however, not always caused by external insults but may arise from inherited genetic defects in pathways regulating IL-1β and/or IL-18 expression and release. Given the absence of external stimuli, these pathologies have been termed autoinflammatory diseases. Monogenic autoinflammatory diseases are a group of rare hereditary syndromes with early manifestation in childhood. They present as inflammatory recurrent flares of fever and skin lesions due to the uncontrolled production of IL-1β and IL-18.

Cryopyrin-associated periodic syndromes (CAPS) are disorders caused by mutations in the *NLRP3* gene, previously known as cold-induced autoinflammatory syndrome 1, which results in uncontrolled processing of IL-1β and IL-18. CAPS is a spectrum of three syndromes of increasing severity: familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS) and chronic infantile neurological cutaneous and articular syndrome (CINCA) also known as neonatal-onset multisystem inflammatory disease (NOMID). Clinically, these 3 syndromes share episodes of recurring fever, urticaria-like skin-lesions, conjunctivitis and inflammatory joint pain. In MWS and CINCA, progressive hearing loss and eye inflammation occur; in CINCA, the most severe form of CAPS, central nervous system inflammation is the most devastating symptom leading to increased intracranial pressure and aseptic meningitis (Goldbach-Mansky, 2011). The mutations in the *NLRP3* gene causing FCAS, MWS and CINCA were identified before the discovery of the inflammasome (Hoffman et al., 2001; Aksentijevich et al., 2002). To date, 182 mutations in the *NLRP3* gene have been reported in the online *registry of hereditary autoinflammatory disorders mutations* (Infevers, 2017). Mouse models for FCAS and MWS were generated by knocking-in NLRP3 with a L351P and A350V mutation, respectively. Mating of these mice with *Il1r1*$^{-/-}$ mice confirmed the pivotal role of IL-1β in the pathogenesis of these diseases but did not completely rescue the phenotype suggesting a possible IL-18

involvement (Brydges et al., 2009). Generation of FCAS mice lacking both IL-1 and IL-18 receptors did not prevent the mice from succumbing to the disease; this could be explained by residual inflammation due to increased pyroptosis (Brydges et al., 2013). Although mutations in *NLRP3* gene are the major cause of CAPS, mutation in NLRC4 and NLRP12 have also been reported in few cases (Jeru et al., 2008; Kitamura et al., 2014).

Familial Mediterranean fever (FMF) is an autosomal recessive disorder caused by gain-of-function mutations in the *MEFV* gene encoding pyrin (French, 1997). Pyrin contains a 14-3-3 binding motif which, when phosphorylated, regulates the compartmentalization (Jeru et al., 2005) and inhibits the activity of pyrin (Park et al., 2016). Pyrin mutations or inactivation of effector kinases by bacterial toxins leave the protein unphosphorylated and free to form a pyrin-inflammasome and activate caspase-1 (Chae et al., 2006). The current first-line treatment for FMF is colchicine, which, via RhoA effector kinases, can lead to pyrin phosphorylation and result in its inactivation (Park et al., 2016). Symptoms of FMF include periodic fever attacks, abdominal and chest pain, serositis, amyloidosis and cutaneous inflammation (Jesus and Goldbach-Mansky, 2014). Recently, a specific dominantly inherited S242R mutation in the 14-3-3 binding motif has been identified and shown to result in pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND), an autoinflammatory disease with distinct clinical features such as severe recurrent neutrophilic dermatosis, fever and absence of serositis and amyloidosis (Masters et al., 2016).

Deficiency of IL-1 receptor antagonist (DIRA) is very rare autoinflammatory disease with onset in the neonatal period and presents as systemic inflammation, pustular skin lesions, joint swelling, periostitis and multifocal osteomyelitis (Altiok et al., 2012). DIRA is caused by homozygous mutations in the *IL1RN* gene. It was first described in nine children harboring mutations leading to the synthesis of a truncated non-functional form of IL-1Ra (Aksentijevich et al., 2009). Around the same time, another group reported the case of a 49-day-old baby presenting a 175-kb homozygous deletion in chromosome 2 which was spaced over six IL-1 family members including *IL1RN* (Reddy et al., 2009).

Mevalonate kinase deficiency (MKD) is an autosomal recessive metabolic disorder caused by mutations in the *MVK* gene (Haas and Hoffmann, 2006). Mevalonate kinase is an enzyme involved in the synthesis of cholesterol and isoprenoids. Mutations in this gene lead to shortage of geranylgeranylated proteins which causes the activation of the pyrin inflammasome and subsequent secretion of IL-1β (Mandey et al., 2006; van der Burgh et al., 2013; Park et al., 2016). Two forms of the disease exist. The less severe hyperimmunoglobulinemia D syndrome (HIDS) is characterized by sporadic fever episodes with skin lesions (widespread erythematous macules and papules), lymphadenopathy, abdominal and joint pain, diarrhea and headache (van der Meer et al., 1984). The rare, more severe form of the disease mevalonic aciduria (MVA) presents all above symptoms chronically (Berger et al., 1985).

Other monogenic autoinflammatory diseases include tumor necrosis factor receptor associated periodic Syndrome (TRAPS), the pyogenic arthritis, pyoderma gangrenosum, and acne (PAPA) syndrome and

Blau syndrome (BS). TRAPS is an autosomal dominant inherited disorder linked to mutations of *TNFRSF1A* gene encoding the TNFα receptor 1 (McDermott et al., 1999; Hull et al., 2002). These mutations produce a misfolded receptor defective in shedding that accumulates in the cytoplasm and results in enhanced NF-κB activation, ROS production and impaired autophagy (Bachetti and Ceccherini, 2014). PAPA syndrome is a hereditary autosomal dominant autoinflammatory syndrome caused by gain-of-function mutations in the *PSTPIP1* gene (Lindor et al., 1997; Wise et al., 2002). The resulting mutated protein interacts with, and activates pyrin, causing dysregulated processing of IL-1β and IL-18 (Shoham et al., 2003). Blau syndrome is an autosomal dominant granulomatous disease caused by a mutation in *CARD15/NOD2* gene (Miceli-Richard et al., 2001). NOD2 is an intracellular receptor able to sense the bacterial peptidoglycan muramyl dipeptide (MDP) (McDermott et al., 1999), and its mutation results in increased NF-κB activity (Maekawa et al., 2016). Moreover, neutrophilic dermatoses such as pyoderma gangrenosum and Sweet's syndrome are also linked to IL-1β overexpression (Marzano et al., 2014; Imhof et al., 2015; Kolios et al., 2015).

Given the key role of IL-1β in inflammatory and autoinflammatory disorders, several IL-1 inhibitors have been developed and evaluated especially in life-threatening autoinflammatory syndromes. To date, the most efficient way to block IL-1 signaling consists of biologics that specifically target IL-1 or IL-1R1. The three main IL-1 antagonists employed as a treatment are Anakinra, Rilonacept and Canakinumab. Anakinra (Kineret®; Sobi, Inc.) is a recombinant non-glycosylated homolog of IL-1Ra that competes with both IL-1α and IL-1β for the binding to IL-1R1 thus impairing the recruitment of IL-1RaP and downstream NF-κB/MAPKs signaling. Rilonacept (Arcalyst®; Regeneron) is a long-acting dimeric fusion protein consisting of portions of IL-1R1 and IL-1RaP linked to the Fc portion of human immunoglobulin G1 (IgG1). Rilonacept acts a soluble decoy binding IL-1β, but also IL-1α and IL-Ra, therefore inhibiting their association with cell surface receptors (IL-1Trap). Rilonacept binds 3 times stronger to IL-1β than to IL-1α and 12 times stronger to IL-1β than to IL-1Ra. Canakinumab (ACZ885, Ilaris®; Novartis) is a human anti-IL1β monoclonal IgG1/κ isotype antibody with a terminal half-life of 23-26 days. The introduction of IL-1 antagonists represents a major breakthrough in the management of several autoinflammatory diseases and their clinical responses suggest that IL-1β plays a critical role in the pathogenesis of autoinflammatory disorders. Indeed, many studies have demonstrated that there is no loss in therapeutic efficacy when Anakinra is substituted with the IL-1β-specific antagonist Canakinumab, suggesting that in comparison to IL-1α and/or IL-18, IL-1β likely plays a predominant role in these diseases.

1.3 MITOGEN-ACTIVATED PROTEIN KINASES

Extracellular signal-regulated kinase (ERK), Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK) family members are well described kinases involved in major physiological pathways by regulating gene transcription, protein biosynthesis, and controlling cell cycle,

differentiation and apoptosis (Chang and Karin, 2001). MAPKs are found in all eukaryotic cells and are activated by numerous environmental stresses, inflammatory mediators such as growth factors, cytokines, and hormones (Arthur and Ley, 2013). It is known that most bacterial infections activate MAPKs, whereas certain bacteria have developed means to inhibit MAPK signaling for their survival (Broz and Monack, 2011; Krachler et al., 2011). Activation of MAPKs is a signaling cascade involving at least three kinases (Figure 8): MAPK kinase kinases (MAP3Ks) get activated by phosphorylation by upstream kinases or interactions with signaling adaptors and small GTPases. MAP3Ks phosphorylate then MAPK kinases (MAP2Ks). These, in turn, dually phosphorylate MAPKs on both threonine and tyrosine residues of the Thr-x-Tyr motif. ERKs, JNKs and p38s are serine/threonine kinases and preferentially phosphorylate residues followed immediately by proline (Kyriakis and Avruch, 2012). The remarkable substrate selectivity between subgroups is achieved by direct interaction of the kinases with specific MAPK docking sites on their bona fide substrates (Tanoue and Nishida, 2003).

MAPKs are known to play important roles in cutaneous inflammation in response to sterile stressors and pathogenic microorganisms. For instance, UV-related stress immediately activates MAPKs and their involvement in the pathogenesis of pemphigus and skin tumorigenesis has been suggested (Mavropoulos et al., 2013; Muthusamy and Piva, 2013; Liu et al., 2014).

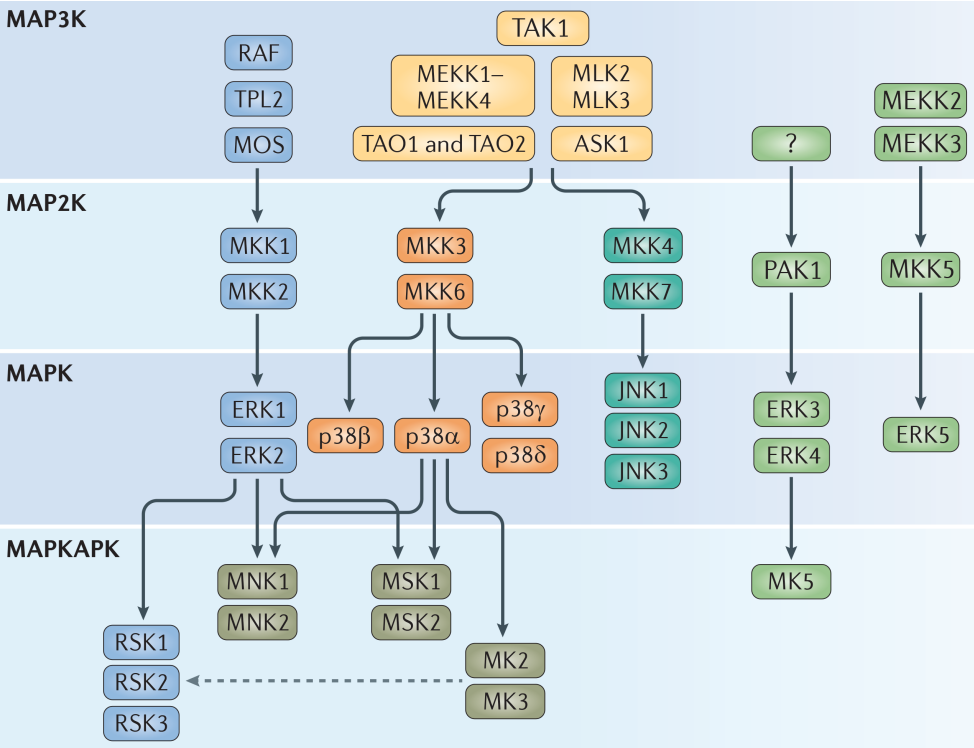

Figure 8 The MAPKs signaling cascade

ERKs, JNKs and p38 MAPKs are activated in a signaling cascade by specific MAP2Ks and MAPK3Ks. ERKs and p38 MAPKs share the MAPK activated protein kinases (MAPKAPK) MNKs and MSKs. The MAP3K TAK1 can activate both MKK3/6 and MKK4/7 resulting in both JNKs and p38 MAPKs activation. Adapted from (Arthur and Ley, 2013).

EXTRACELLULAR SIGNAL-REGULATED KINASE (ERK)

The extracellular signal-regulated kinase subgroup includes two isoforms: ERK1 (MAPK3) and ERK2 (MAPK1). ERK3/4, ERK5 and ERK7/8 form separate subgroups since they have independent activation pathways and different targets. ERK1/2 were the first mammalian MAPKs described (Seger and Krebs, 1995). They are involved in several cellular functions such as mobility, migration, proliferation, differentiation, cytoskeletal polymerization, transcription regulation and development (Yoon and Seger, 2006). Activation of the GTPase Ras by mitogens leads to the initiation of the ERK signaling cascade which involves the MAP3K Raf and the MAP2Ks MEK1/2. Activated ERK1/2 undergo homodimerization (Casar et al., 2008) and further trigger downstream cytoplasmic substrates such as ribosomal S6 kinases (RSKs), MAPK interacting kinase (MNK) and mitogen- and stress-activated kinases (MSKs) (Arthur and Ley, 2013). Moreover, ERKs translocation in the nucleus leads to the activation of transcription factors including Activator Protein-1 (AP-1) and c-Myc. ERK1/2 share with p38 MAPKs the downstream activated protein kinases (MAPKAPK) MNKs and MSKs, involved in mRNA translation via eukaryotic translation initiation factor 4G (eIF4G) (Waskiewicz et al., 1997) and activation of the transcription factors ATF1 and cyclic AMP response element-binding protein (CREB) (Wiggin et al., 2002). Dysregulation of the ERK signaling pathway has been associated with a high frequency of human cancers including melanoma, a malignant tumor originating from melanocytes (Dhillon et al., 2007). Every member of the pathway has been reported to be mutated: mutations of the GTPase Ras were the first to be reported (Ball et al., 1994) and *NRAS* gene mutations account for 15–20% of all melanomas (Johnson and Puzanov, 2015), whereas mutations of the MAP3K BRAF drive 50% of melanomas (Ascierto et al., 2012).

JUN N-TERMINAL KINASE (JNK)

First described as stress-activated protein kinases (SAPKs) (Kyriakis and Avruch, 2012), the Jun N-terminal kinase (JNK) subgroup comprises three members: JNK1 (MAPK8) and JNK2 (MAPK9) are ubiquitously expressed in all cells and tissues, whereas JNK3 (MAPK10) is mainly found in the brain, testes and cardiac myocytes (Xie et al., 1998). JNKs have in total 11 isoforms: different mRNA processing results in the translation of light 46 kDa and heavy 55 kDa JNK1 and JNK2. Due to alternative splicing, they have two additional isoforms for a total of four each. Alternative translation initiation and splicing of the JNK3 mRNA results instead in 3 isoforms (Zeke et al., 2016). JNKs isoforms show more than 70% amino acid sequence identity (Park et al., 2015) and they are activated by dual phosphorylation of their Thr-Pro-Tyr motif by the MAP2Ks MKK4 and MKK7 which in turn are triggered by MAP3Ks such as TAK1 and apoptosis signal-regulating kinase 1 (ASK1) (Arthur and Ley, 2013). The JNK signaling pathway is activated by a variety of proinflammatory cytokines, such as TNFα via the ASK1/MKK7 axis or IL-1 via TRAF6/TAK1. Recognition of cognate ligands by TLRs also triggers the MAP3K TAK1 which initiates a signaling cascade that culminates not only with

activation of JNKs but also p38 and NF-κB (Dai et al., 2012). Indeed, TAK1 can phosphorylate MKK4/7 but also MKK3/6 and activate the IκB kinase (IKK) complex (Wang et al., 2001). Furthermore, TAK1 is also able to activate the less described MAPK family member NEMO-like kinase (NLK) involved in development, proliferation and apoptosis regulation (Ishitani et al., 2003; Liu et al., 2016). Other stimuli such UV light and heat shock or growth factors are activators of the JNK pathway (Adler et al., 1995; Nakamura et al., 2001). Once activated, JNKs migrate to the nucleus where they phosphorylate numerous transcription factors like the AP-1 component c-Jun but also p53, NFAT, STAT1/3, ATF2, thus regulating gene expression and cellular activities such as differentiation (Bogoyevitch and Kobe, 2006). Furthermore, JNKs play a role in the intrinsic apoptotic pathway by phosphorylating Bcl-2 family members (Lei and Davis, 2003; Kim et al., 2006).

P38 MITOGEN-ACTIVATED PROTEIN KINASE (P38 MAPK)

The p38 MAPKs are stress-activated kinases that, similarly to JNKs, are activated by several extracellular stresses such as UV light, heat and osmotic shock but also by the proinflammatory cytokines TNFα and IL-1 (Zarubin and Han, 2005). As suggested by their name, p38 MAPKs were first identified as a 38 kDa protein that is phosphorylated upon LPS exposure (Han et al., 1993). Four isoforms encoded by different genes exist: p38α (MAPK14) and p38β (MAPK11) are expressed everywhere, while p38γ (MAPK12) is mainly expressed in skeletal muscles and in the brain (Li et al., 1996) and p38δ (MAPK13) is expressed in testes, pancreas and small intestine (Ashwell, 2006). Additionally, there are three alternatively splice isoforms: p38α variants Mxi2 and Exip (Zervos et al., 1995; Sudo et al., 2002), and p38β variant p38β-2 (Wang et al., 2000). The p38α/β and p38γ/δ isoform pairs have 74% and 68% amino acid sequence identity, respectively, and only around 60% between them four (Jiang et al., 1997). p38 MAPKs are canonically activated by dual phosphorylation on their Thr-Gly-Tyr motif by the MAP2Ks MKK3 and MKK6. Furthermore, the JNK's MKK4 was also described to phosphorylate and activate p38 (Derijard et al., 1995; Brancho et al., 2003). Once activated, p38 MAPKs can translocate to the nucleus and regulate the expression of genes involved in inflammation, apoptosis, growth and differentiation by triggering several transcription factors including ATF1/2, p53 and NFAT (Hazzalin et al., 1996; Tan et al., 1996; Huang et al., 1999; Gomez del Arco et al., 2000). Similarly to JNKs, p38 MAPKs are able to phosphorylate the anti-apoptotic protein Bcl-2 resulting in the release of cytochrome c from the mitochondria (De Chiara et al., 2006). p38 MAPKs have several cytosolic targets such as MNKs and MSKs (shared with ERK1/2) and MK2/3. Effector kinases MK2/3 are activated by p38α and p38β and are involved in the regulation of gene expression at the post-transcriptional level by mRNA stabilization. MK2 can phosphorylate tristetraprolin (TTP), a mRNA-AU-rich-element (ARE)-binding protein, thus impairing dephospho-TTP-dependent mRNA degradation (Gaestel, 2006). Moreover, MK2 also phosphorylates human antigen R (HuR) and heterogeneous nuclear ribonucleoprotein A0 (hnRNP-A0), two proteins that directly bind to and

stabilize mRNA (Kyriakis and Avruch, 2012). MK2/3 also target the heat shock protein 27 (Hsp27) involved in actin reorganization and cell motility (Huot et al., 1997). Moreover, p38α is involved in the phosphorylation of keratins 8 and 19 in epithelial cells (Feng et al., 1999) and p38δ regulates the expression of the structural protein involucrin during keratinocyte differentiation (Efimova et al., 2003). Increased levels of phosphorylated p38 have been reported in psoriatic skin (Yu et al., 2007). The abnormal activity of the kinases results in the production of proinflammatory cytokines such as TNFα and IL-17A which induces the expression of cathelicidin antimicrobial peptide, β-defensin and S100A8 (Hau et al., 2013; Mose et al., 2013).

INFLAMMASOME REGULATION BY MAPKS AND OTHER KINASES

MAPKs play fundamental roles in innate immunity; indeed, recognition of pathogens by PRRs such as TLRs causes the activation of the JNK, p38 and NF-κB pathway in a TAK1-dependent mechanism. JNKs, mainly JNK1, are also associated with the activation of the NLRP3 and AIM2 inflammasomes in murine hematopoietic immune cells by rearrangement of the inflammasome adaptor ASC around the nucleus (Hara et al., 2013; Okada et al., 2014). Phosphorylation on ASC tyrosine 144 residue is crucial for oligomerization and recruitment of caspase-1 but evidence of a direct phosphorylation by JNKs is missing (Hara et al., 2013).

Other kinases such as NEK7, Syk, Lyn, FAK/Pyk2, BTK, PKC-δ, PKR, PKNs, IKKα and IRAK-1, have been shown to be involved in the regulation of inflammasome activation. NEK7, a member of the NIMA (never in mitosis gene A)-related serine-threonine kinase family, was shown to directly interact with the LRR domain of NLRP3 in response to ROS production and potassium efflux following stimulation with nigericin, ATP or alum. Interestingly, its kinase activity is not required for the activation of the inflammasome suggesting a possible role as mediator of inflammasome assembly (He et al., 2016; Schmid-Burgk et al., 2016; Shi et al., 2016). Also, activated double-stranded RNA-dependent protein kinase (PKR) was reported to bind to NLRP1, NLRP3, NLRC4 and AIM2 and trigger their activation (Lu et al., 2012). The spleen tyrosine kinase (Syk) has been described to drive phosphorylation and spatial redistribution of the inflammasome adaptor ASC, similarly to JNK, thus favoring its oligomerization (Hara et al., 2013; Lin et al., 2015). Moreover, Syk is also implicated in NLRP3 activation upon sensing of fungal infection by dectin-1 (Gross et al., 2009; Kistowska et al., 2014a) or jointly with the tyrosine-protein kinase Lyn upon malarial hemozoin challenge (Shio et al., 2009). Focal adhesion kinase (FAK) and proline-rich tyrosine kinase 2 (Pyk2) are two kinases involved primarily in cell migration. Nigericin treatment was shown to induce the Syk-mediated phosphorylation of FAK and Pyk2 which drives the interaction with ASC and its oligomerization. Furthermore, p-Pyk2 is able to phosphorylate human ASC at the tyrosine 146 residue (Chung et al., 2016a). Bruton's tyrosine kinase (BTK) is a kinase primarily involved in B cell maturation but also in TLR signaling (Lee et al., 2012). BTK is activated by Syk and Lyn and it is also able to interact with NLRP3 and ASC thus driving

oligomerization and recruitment of caspase-1 (Ito et al., 2015). Furthermore, protein kinase C δ-type (PKC-δ) has been reported to activate NLRC4 by inducing its phosphorylation at the serine 533 residue upon *Salmonella typhimurium* infection (Qu et al., 2012). More recently, it has been demonstrated that PKC activity is also linked to the ROS-mediated activation of NLRP3 (Shio et al., 2015). Contrarily to other kinases, PKN1 and PKN2 have an inhibitory function. They bind and phosphorylate pyrin promoting its association with 14-3-3 scaffold proteins and segregation in the nucleus (Park et al., 2016). Also, IKKα can interact with ASC, segregating the latter in the nucleus of macrophages. Activation of IKKα releases ASC and allows inflammasome activation (Martin et al., 2014). Finally, IRAK-1, an enzyme participating in Myd88 signaling, has been shown to induce a rapid NLRP3 inflammasome activation without priming requirement (Lin et al., 2014).

Inflammasome activation is a complex and tightly controlled process in which kinases play a significant role. It is therefore of interest to further investigate the role of MAPKs in this context.

1.5 AIM OF THE THESIS

In 2002, intracellular protein complexes known as the inflammasomes were discovered and shown to have a crucial role in the sensing of intracellular pathogen- and danger-associated molecular patterns (PAMPs and DAMPs). Activation of the inflammasomes results in the processing and subsequent secretion of the pro-inflammatory cytokines IL-1β and IL-18. Extracellular signal-regulated kinase (ERK), Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK) family members are activated by numerous environmental stresses and are involved in gene transcription and protein biosynthesis, regulating major physiological pathways such as cell differentiation, apoptosis and inflammation.

The aim of this thesis was to investigate of the role of MAPKs, especially JNK and p38, in the activation of the inflammasome in immune cells and in primary keratinocytes. Soon after the beginning of this thesis, a series of studies describing the involvement of JNK in NLRP3 activation in macrophages were released. We therefore decided to focus on the role of p38 in keratinocytes since the mechanisms regulating inflammasome activation in these cells has never been investigated to date.

Furthermore, the need for a clean method to completely abrogate gene expression in keratinocytes, together with the curiosity of learning and applying a new method, led us to establish a protocol to target primary keratinocytes with the CRISPR/Cas9 technology.

CHAPTER 2: MATERIALS AND METHODS

GENERAL REMARKS

Material and methods are adapted from the dissertations of Dr. Gerhard Strittmatter (ETH Diss.-No 22564) Standard methods are described only briefly, but modifications of commonly used protocols are stated. Deviations from protocols from single experiments can be found in the respective figure legends.

2.1 MATERIALS

CHEMICALS AND OTHER CONSUMABLES

Acetic acid	Fluka Chemie, Buchs, Switzerland
Acetone	Merck, Darmstadt, Germany
Acrylamide/bisacrylamide (30:0.8)	ROT, Karlsruhe, Germany
Adenosine triphosphate (ATP)	Sigma-Aldrich, Munich, Germany
Agar	Difco, Detroit, US-MI
Agarose	Axon Lab AG, Baden, Switzerland
Ammonium persulfate (APS)	Sigma-Aldrich, Munich, Germany
Ammonium pyrrolidinedithiocarbamate (PDTC)	Sigma-Aldrich, Munich, Germany
Ampicillin	Sigma-Aldrich, Munich, Germany
Antibody Diluent	DAKO, Glostrup, Denmark
Antimycin A	Sigma-Aldrich, Munich, Germany
apo-Transferrin	Sigma-Aldrich, Munich, Germany
Bovine serum albumin (BSA)	Sigma-Aldrich, Munich, Germany
Bromophenol blue	Sigma-Aldrich, Munich, Germany
BSA Fraction V	PAA, Pasching, Austria
Calcium chloride ($CaCl_2$)	Fluka Chemie, Buchs, Switzerland
Chloroform	Fluka Chemie, Buchs, Switzerland
Citric acid	Fluka Chemie, Buchs, Switzerland
Deoxynucleotide triphosphates (dNTPs)	Roche, Rotkreuz, Switzerland
Diethylpyrocarbonate (DEPC)	Sigma-Aldrich, Munich, Germany
Dimethylsulfoxide (DMSO)	Merck, Darmstadt, Switzerland
di-Potassium hydrogen phosphate (K_2HPO4)	Fluka Chemie, Buchs, Switzerland
Dipotassium phosphate (K_2HPO_4)	Fluka Chemie, Buchs, Switzerland
Dithiothreitol (DTT)	Sigma-Aldrich, Munich, Germany
Disuccinimidyl suberate (DSS)	Thermo Fisher Scientific, Waltham, US-MA
DNA Gel Loading Dye	Thermo Fisher Scientific, Waltham, US-MA
Ethanol (EtOH)	Fluka Chemie, Buchs, Switzerland
Ethanol 70% V/V	Kantonsapotheke, Zurich, Switzerland

Ethanol 80% V/V KA PhEur	Kantonsapotheke, Zurich, Switzerland
Ethanol 96% V/V KA PhEur	Kantonsapotheke, Zurich, Switzerland
Ethanol absolut KA PhEur	Kantonsapotheke, Zurich, Switzerland
Ethanolamine	Sigma-Aldrich, Munich, Germany
Ethidium Bromide	Sigma-Aldrich, Munich, Germany
Ethylenediaminetetraacetic acid (EDTA)	Fluka Chemie, Buchs, Switzerland
FastStart Universal Sybr Green Master	Roche, Rotkreuz, Switzerland
Ficoll-Paque PLUS	GE Healthcare, Little Chalfont, UK
Flasks and (multiwell) dishes for cell culture	NUNC, Roskilde, Denmark
GelGreen	Biotium, Hayward, US-CA
Glybenclamide	Sigma-Aldrich, Munich, Germany
Glycerol	Merck, Darmstadt, Germany
Glycine	ROT, Karlsruhe, Germany
HEPES	Sigma-Aldrich, Munich, Germany
Hydrogen chloride (HCl)	Fluka Chemie, Buchs, Switzerland
Hydrogen peroxide (H_2O_2, 30%)	Merck, Darmstadt, Germany
Imidazole	Merck, Darmstadt, Germany
Isotonic saline	B. BRAUN, Emmenbrücke, Switzerland
Ketamine	Veterinaria, Zürich, Switzerland
Lipopolysaccharide (LPS)	Fluka Chemie, Buchs, Switzerland
Luminol	Fluka Chemie, Buchs, Switzerland
Magnesium chloride ($MgCl_2$)	Fluka Chemie, Buchs, Switzerland
Methanol	Fluka Chemie, Buchs, Switzerland
Microfilter units (0.22/0.45 µm)	Millipore, Billerica, US-MA
Milk powder (low fat)	Migros, Zürich, Switzerland
Nigericin	Selleck Chemicals, Houston, US-TX
Nitrocellulose membrane (Protran 0.2 µm)	GE Healthcare, Little Chalfont, UK
Nonidet P-40	Sigma-Aldrich, Munich, Germany
O-Phosphorylethanolamine	Sigma-Aldrich, Munich, Germany
Pan-caspase inhibitor (z-VAD-fmk)	Alexis, Lausen, Switzerland
Paraformaldehyde (PFA)	Sigma-Aldrich, Munich, Germany
Phosphatase inhibitor cocktail	Bimake, Houston, US-TX
Potassium chloride (KCl)	Fluka Chemie, Buchs, Switzerland
ProLong Gold mounting medium	Thermo Fisher Scientific, Waltham, US-MA
Proteases inhibitor cocktail	Bimake, Houston, US-TX
Protein A sepharose (PAS)	Amersham, Uppsala, Sweden

Slides Super Frost	SHANDON, Frankfurt, Germany
Sodium chloride (NaCl)	Fluka Chemie, Buchs, Switzerland
Sodium dihydrogen phosphate (NaH_2PO_4)	Fluka Chemie, Buchs, Switzerland
Sodium dodecyl sulfate (SDS)	Sigma-Aldrich, Munich, Germany
Sodium hydrogen phosphate (Na_2HPO_4)	Fluka Chemie, Buchs, Switzerland
Sodium hydroxide (NaOH)	Fluka Chemie, Buchs, Switzerland
Sucrose	Sigma-Aldrich, Munich, Germany
Sulfuric acid (H_2SO_4)	Fluka Chemie, Buchs, Switzerland
Tetramethylethylenediamide (TEMED)	Sigma-Aldrich, Munich, Germany
Triton-X 100	Sigma-Aldrich, Munich, Germany
TRIZMA Base (Tris)	Sigma-Aldrich, Munich, Germany
Tween 20	ROT, Karlsruhe, Germany
Ultracentrifugation units (Amicon)	Millipore, Billerica, US-MA
Ultrafiltration units (Amicon)	Millipore, Billerica, US-MA
Ultrapure LPS (upLPS)	InVivogen, Toulouse, France
Uric acid	Sigma-Aldrich, Munich, Germany
Whatman 3MM paper	Whatman, Maidstone, England
Xylazine	Veterinaria, Zürich, Switzerland
Yeast extract	Difco, Detroit, US-MI
β-mercaptoethanol	Sigma-Aldrich, Munich, Germany

SMALL CHEMICAL INHIBITORS

BIRB 796	Selleck Chemicals, Houston, US-TX
MCC950	Selleck Chemicals, Houston, US-TX
p38 Inhibitor I (506126)	Merck Millipore, Billerica, US-MA
Z-VAD-fmk	Selleck Chemicals, Houston, US-TX
SB203580	Selleck Chemicals, Houston, US-TX
SP600125	Selleck Chemicals, Houston, US-TX
Piceatannol	Sigma-Aldrich, Munich, Germany
U0126	Sigma-Aldrich, Munich, Germany
VX-702	Selleck Chemicals, Houston, US-TX
Z-VAD-fmk	Selleck Chemicals, Houston, US-TX

PROTEIN SIZE STANDARDS

Prestained protein MW marker	Thermo Fisher Scientific, Waltham, US-MA
Prestained Protein Ladder V	Geneaid, Taipei, Taiwan

DNA SIZE STANDARDS

GeneRuler DNA ladder mix	Thermo Fisher Scientific, Waltham, US-MA
GeneRuler 100 bp DNA ladder	Thermo Fisher Scientific, Waltham, US-MA
GeneRuler 50 bp DNA ladder	Thermo Fisher Scientific, Waltham, US-MA

CELL CULTURE MEDIA AND ADDITIVES

Anti-anti antibiotic-antimycotic (A/A)	GIBCO BRL, Paisley, Scotland
Blasticidin S hydrochloride	Sigma-Aldrich, Munich, Germany
DMEM (order no. 41966-029)	GIBCO BRL, Paisley, Scotland
Doxycycline	Sigma-Aldrich, Munich, Germany
EDTA	GIBCO BRL, Paisley, Scotland
Fetal calf serum (FCS), heat inactivated	GIBCO BRL, Paisley, Scotland
GlutaMAX	GIBCO BRL, Paisley, Scotland
Ham's F-12	GIBCO BRL, Paisley, Scotland
KBM CD	Lonza, Basel, Switzerland
Mitomycin c	Sigma-Aldrich, Munich, Germany
Non-essential amino acids (NEAA)	GIBCO BRL, Paisley, Scotland
OptiMEM (order no. 11058-021)	GIBCO BRL, Paisley, Scotland
Penicillin/Streptomycin (P/S)	Sigma-Aldrich, Munich, Germany
Puromycin	Sigma-Aldrich, Munich, Germany
RPMI 1640 (order no. 61870)	GIBCO BRL, Paisley, Scotland
Sodium pyruvate 100 mM	GIBCO BRL, Paisley, Scotland
Keratinocyte-SFM, EGF, BPE (order no. 17005)	GIBCO BRL, Paisley, Scotland

TRANSFECTION REAGENTS

INTERFERin	PolyPlus, Illkirch, France
Lipofectamine 2000	Invitrogen, Basel, Switzerland
TransIT-X2	Mirus Bio Lcc, Madison, US-WI

KITS

BCA protein assay	Pierce, Rockford, US-IL
CytoTox 96 LDH assay kit	Promega, Madison, US-WC
Gel extraction/PCR purification kit	Macherey-Nagel, Düren, Germany
GenoPure Plasmid Midi/Maxi	Roche, Rotkreuz, Switzerland
High Pure Plasmid Isolation kit	Roche, Rotkreuz, Switzerland
High Pure RNA Isolation Kit	Roche, Rotkreuz, Switzerland
KAPA2G Fast HotStart PCR Kit	Kapa Biosystems, Wilmington, US-MA
NBT/BCIP substrate kit	Promega, Madison, US-WC
RevertAid First Strand cDNA Synthesis Kit	Thermo Fisher Scientific, Waltham, US-MA
TMB substrate kit	Pierce, Rockford, US-IL

ENZYMES

Accutase	PAA, Parsching, Austria
Calf intestinal phosphatase (CIP)	NEB, Ipswich, US-MA
Dispase II	Roche, Rotkreuz, Switzerland
DNA-restriction enzymes	NEB, Ipswich, US-MA
DNA-restriction enzyme buffers	NEB, Ipswich, US-MA
Gateway LR Clonase II Enzyme mix	Life Technologies, Carlsbad, US-CA
Pfu DNA polymerase	Thermo Fisher Scientific, Waltham, US-MA
Proteinase K	Roche, Rotkreuz, Switzerland
T4 DNA ligase	Roche, Rotkreuz, Switzerland
Taq DNA polymerase	Thermo Fisher Scientific, Waltham, US-MA
Trypsin	GIBCO BRL, Paisley, Scotland

PRIMARY ANTIBODIES

Antibody clone number / manufacturers' order number is indicated in parentheses.

anti-ASC (rabbit, polyclonal, AL177)	AdipoGen, Epalinges, Switzerland
anti-ASC (rabbit, polyclonal, N-15R)	Santa Cruz Biotechnology, Santa Cruz, US-CA
anti-Caspase-1 (CARD, rabbit, polyclonal, A-19)	Santa Cruz Biotechnology, Santa Cruz, US-CA
anti-Flag M2 (mouse, monoclonal, F1804)	Sigma-Aldrich, Munich, Germany
anti-HA (mouse, monoclonal, 1583816)	Roche, Rotkreuz, Switzerland
anti-HA (rabbit, polyclonal, Y-11)	Santa Cruz Biotechnology, Santa Cruz, US-CA
anti-IL-18 (rabbit, polyclonal, PM014)	MBL, Woburn, US-MA
anti-IL-1β (mouse, monoclonal, MAB201)	R&D Systems, Minneapolis, US-MN

anti-JNK1 (mouse, monoclonal, 2C6)	Cell Signaling Technology, Danvers, US-MA
anti-JNK2 (rabbit, monoclonal, 56G8)	Cell Signaling Technology, Danvers, US-MA
anti-Myc (mouse, hybridoma supernatant)	described by (Munding et al., 2006)
anti-Myc (mouse, monoclonal, 631206)	Clontech Labs, Mountain View, US-CA
anti-β-Actin (mouse, monoclonal, A5441)	Sigma-Aldrich, Munich, Germany
anti-NLRP1 (polyclonal, rabbit, AL176)	AdipoGen, Epalinges, Switzerland
anti-NLRP3 (mouse, monoclonal, Cryo-2)	AdipoGen, Epalinges, Switzerland
anti-p38 (rabbit, monoclonal, 8690)	Cell Signaling Technology, Danvers, US-MA
anti-p38α (rabbit, monoclonal, 9218)	Cell Signaling Technology, Danvers, US-MA
anti-p38β (rabbit, monoclonal, 2339)	Cell Signaling Technology, Danvers, US-MA
anti-p38γ (rabbit, monoclonal, 2308)	Cell Signaling Technology, Danvers, US-MA
anti-p38δ (rabbit, monoclonal, 2307)	Cell Signaling Technology, Danvers, US-MA
anti-phospho-JNK (rabbit, polyclonal, 9251)	Cell Signaling Technology, Danvers, US-MA
anti-phospho-p38 (monoclonal, rabbit, 4511)	Cell Signaling Technology, Danvers, US-MA
anti-phospho-tyrosine (P-Tyr-1000, rabbit, monoclonal, 8954)	Cell Signaling Technology, Danvers, US-MA

SECONDARY ANTIBODIES

Manufacturers' order number is indicated in parentheses.

anti-Mouse IgG (AP-conjugated, S372B)	Promega, Madison, US-WC
anti-Rabbit IgG (AP-conjugated, S373B)	Promega, Madison, US-WC
anti-Rabbit IgG (DyLight 488-conjugated, ab96891)	Abcam, Cambridge, UK

ANTIBODIES FOR FLOW CYTOMETRY

Manufacturers' order number is indicated in parentheses.

anti-7/4 (FITC-conjugated, ab53453)	Abcam, Cambridge, UK
anti-CD11b (PE-conjugated, 1012079)	BioLegend, San Diego, US-CA
anti-CD11c (PE-conjugated, HL3)	BD, Franklin Lakes, US-NJ
anti-CD11c (FITC-conjugated, N418)	BioLegend, San Diego, US-CA
anti-CD19 (PerCP-Cy5.5-conjugated, 6D5)	BioLegend
anti-Ly6G (APC-eFluor780-conjugated, RB6, 8C5)	Thermo Fisher Scientific, Waltham, US-MA
anti-F4/80 (APC-conjugated, BM8)	BioLegend, San Diego, US-CA

siRNA

The following 21-mere duplexes were from Sigma-Aldrich (Munich, Germany).

Caspase-1	
Sense	GGC AGA GAU UUA UCC AAU Att
Antisense	UAU UGG AUA AAU CUU GCCga

p38α (MAPK14)	
Sense	GGU CUA AAG UAU AUA CAU Utt
Antisense	AAU GUA UAU ACU UUA GAC Ctt

p38β (MAPK11)	
Sense	CAC GUU CAA UUC CUG GUU Utt
Antisense	AAA CCA GGA AUU GAA CGU Gtt

p38γ (MAPK12)	
Sense	GAG GUC AUC UUG AAU UGG Att
Antisense	UCC AAU UCA AGA UGA CCU Ctt

p38δ (MAPK13)	
Sense	CAG AGA UGC UGA CAG GGA Att
Antisense	UUC CCU GUC AGC AUC UCU Gtt

Scrambled	
Sense	UUC UCC GAC GUG UCA CGU tt
Antisense	ACG UGA CAC GUU CGG AGA Att

The following 21-mere duplexes are part of a screening siRNA library from Thermo Fisher Scientific (Waltham, US-MA).

Target	Sense	Antisense
AIM2	GCAACGUGCUGCACCAAAAtt	UUUUGGUGCAGCACGUUGCtt
	GGAGAUAAGGUUCGACUUAtt	UAAGUCGAACCUUAUCUCCtt
ASC	GGAUGAGCAGUACCAGGCAtt	UGCCUGGUACUGCUCAUCCgt
	GUUUCACACCAGCCUGGAAtt	UUCCAGGCUGGUGUGAAACtg
Caspase-1	GGAAGACUCAUUGAACAUAtt	UAUGUUCAAUGAGUCUUCCaa
	CCACUGAAAGAGUGACUUUtt	AAAGUCACUCUUUCAGUGGtg
ERK1	GGACCGGAUGUUAACCUUUtt	AAAGGUUAACAUCCGGUCCag
	UGAUGGAGACUGACCUGUAtt	UACAGGUCAGUCUCCAUCAgg
ERK2	CAGGGUUCCUGACAGAAUAtt	UAUUCUGUCAGGAACCCUGtg
	CAACCAUCGAGCAAAUGAAtt	UUCAUUUGCUCGAUGGUUGgt
JNK1	GUUGCAAUCAAGAAGCUAAtt	UUAGCUUCUUGAUUGCAACat
	CAAAGAUCCCUGACAAGCAtt	UGCUUGUCAGGGAUCUUUGgt
JNK2	GCAUUCAGCUGGUAUAAUUtt	AAUUAUACCAGCUGAAUGCag
	GGAAAGAGCUAAUUUACAAtt	UUGUAAAUUAGCUCUUUCCat
NLRP1	GUACGAGACUCGGAACAAAtt	UUUGUUCCGAGUCUCGUACaa
	GGUGGAGCUGCAUCACAUAtt	UAUGUGAUGCAGCUCCACCct
NLRP3	GGAGAGACCUUUAUGAGAAtt	UUCUCAUAAAGGUCUCUCCtg
	GCUUUGUCCUCGGUACUCAtt	UGAGUACCGAGGACAAAGCtg
p38α	CCUAAAACCUAGUAAUCUAtt	UAGAUUACUAGGUUUUAGGtc
	GAAGCUCUCCAGACCAUUUtt	AAAUGGUCUGGAGAGCUUCtt
p38β	GCGACUACAUUGACCAGCUtt	AGCUGGUCAAUGUAGUCGCtt
	GAACACGCCCGGACAUAUAtt	UAUAUGUCCGGGCGUGUUCtg
p38γ	CAGUUCCUCGUGUACCAGAtt	UCUGGUACACGAGGAACUGga
	GAAGUAUGAUGACUCCUUUtt	AAAGGAGUCAUCAUACUUCtg
p38δ	AAUGAGGACUGUGAACUGAtt	UCAGUUCACAGUCCUCAUUca
	GGUGUAUCAGAUGCUCAAAtt	UUUGAGCAUCUGAUACACCag
Scrambled	UAACGACGCGACGACGUAAtt	UUACGUCGUCGCGUCGUUAtt
	UCGUAAGUAAGCGCAACCCtt	GGGUUGCGCUUACUUACGAtt

shRNA

shRNA were from Sigma-Aldrich (Munich, Germany) and they are cloned into the lentiviral vector pLKO.1 .

Lamin A/C
CCGGGAAGCAACTTCAGGATGAGATCTCGAGATCTCATCCTGAAGTTGCTTCTTTTTG

Caspase-1
CCGGATGAGGGCAAGAGCGATTAAACTCGAGTTTAATCGCTCTTGCCCTCATTTTTTG

JNK1
CCGGCAGTAAGGACTTACGTTGAAACTCGAGTTTCAACGTAAGTCCTTACTGTTTTTTG

JNK2
CCGGCTAACTTATGTCAGGTTATTCCTCGAGGAATAACCTGACATAAGTTAGTTTTTTG

sgRNA

These DNA oligos were annealed and cloned into the pLentiCRISPRv2 plasmid. shRNA sequences are bolded.

Control (non-targeting)	
ssDNA Forward	CACCG**GTAGCGAACGTGTCCGGCGT**
ssDNA Reverse	aaacACGCCGGACACGTTCGCTACG

Caspase-1	
ssDNA Forward	CACCG**ATTGACTCCGTTATTCCGAA**
ssDNA Reverse	aaacTTCGGAATAACGGAGTCAATC

ASC	
ssDNA Forward	CACC**GTAGAAGCTGACCAGCTTGT**
ssDNA Reverse	aaacACAAGCTGGTCAGCTTCTAC

NLRP1	
ssDNA Forward	CACC**GCTCAGCCAGAGAAGACGAG**
ssDNA Reverse	aaacCTCGTCTTCTCTGGCTGAGC

NLRP3

ssDNA Forward	CACCG**ATTGAAGTCGATCATTAGCG**
ssDNA Reverse	aaacCGCTAATGATCGACTTCAATC

p38α-1

ssDNA Forward	CACC**GCTGAACAAGACAATCTGGG**
ssDNA Reverse	aaacCCCAGATTGTCTTGTTCAGC

p38α-2

ssDNA Forward	CACCG**CTTATCTACCAAATTCTCCG**
ssDNA Reverse	aaacCGGAGAATTTGGTAGATAAGC

p38β

ssDNA Forward	CACC**GCTGAAGTCCTCGATGGACG**
ssDNA Reverse	aaacCGTCCA TCGAGGACTTCAGC

p38γ

ssDNA Forward	CACC**GCTTGAGCAGGCGCAGCTCG**
ssDNA Reverse	aaacCGAGCTGCGCCTGCTCAAGC

p38δ

ssDNA Forward	CACC**GCGAAGATCTCGGACTGAAA**
ssDNA Reverse	aaacTTTCAGTCCGAGATCTTCGC

PRIMERS

Primers for mouse genotyping

Caspase-1 ko	
Forward	CTG TGG TGA CTA ACC GAT AA
Reverse wildtype	GCG CCT CCC CTA CCC GG
Reverse ko	CAT GCC TGA ATA ATG ATC ACC

JNK1 (MAPK8) ko	
Common	CCA GGC TCT CCT CAT CTT CA
Reverse wildtype	TCA CCA CAT AAG GCG TCA TC
Mutant	CCA GCT CAT TCC TCC ACT CAT G

JNK2 (MAPK9) ko	
Common	GGA GCC CGA TAG TAT CGA GTT ACC
Forward wildtype	GTT AGA CAA TCC CAG AGG TTG TGT G
Forward mutant	CCA GCT CAT TCC TCC ACT CAT G

Primers for real-time PCR (human)

ASC	
Forward	CGC GAG GGT CAC AAA CGT
Reverse	TGC TCA TCC GTC AGG ACC TT

Caspase-1	
Forward	TCC CTA GAA GAA GCT CAA AGG ATA TG
Reverse	CGT GTG CGG CTT GAC TTG

IL-18	
Forward	GCT GCT GAA CCA GTA GAA GAC
Reverse	CCG ATT TCC TTG GTC AAT GAA GA

IL-1β	
Forward	CAC GAT GCA CCT GTA CGA TCA
Reverse	GTT GCT CCA TAT CCT GTC CCT

NLRP1	
Forward	ATC CCT CCA GCC CGC ATA
Reverse	GGT CCA CAA AGT GCA GCA ACT

NLRP3	
Forward	GCA AAA AGA GAT GAG CCG AAG T
Reverse	GCT GTC TTC CTG GCA TAT CAC A

RPL27	
Forward	TCA CCT AAT GCC CAC AAG GTA
Reverse	CCA CTT GTT CTT GCC TGT CTT

Primers for real-time PCR (mouse)

ASC	
Forward	CTT GTC AGG GGA TGA ACT CAA AA
Reverse	GCC ATA CGA CTC CAG ATA GTA GC

Caspase-1	
Forward	ACA AGG CAC GGG ACC TAT G
Reverse	TCC CAG TCA GTC CTG GAA ATG

IL-1β	
Forward	GCA ACT GTT CCT GAA CTC AAC T
Reverse	ATC TTT TGG GGT CCG TCA ACT

JNK1	
Forward	GTGGGGTATGCCCAAGAGG
Reverse	GCCATAAAGCCCAGATAGAGC

JNK2	
Forward	AGTGACAGTAAAAGCGATGGTC
Reverse	AGCACAAACAATTCCTTGGGC

NLRP3	
Forward	ATT ACC CGC CCG AGA AAG G
Reverse	TCG CAG CAA AGA TCC ACA CAG

RPL27	
Forward	AAA GCC GTC ATC GTG AAG AAC
Reverse	GCT GTC ACT TTC CGG GGA TAG

PLASMIDS

pcDNA3	Invitrogen, Basel, Switzerland
pCG	(Beer et al., 2002)
pCMV	CLONTECH, Palo Alto, US-CA
pMD2-VSVG	Prof. Tschopp, University of Lausanne
pSP-93	Oligoengine, Seattle, US-WA
psPAX2	Prof. Tschopp, University of Lausanne
pcDNA3 Flag MKK7B2 Jnk1a1	Addgene: 19726 (Lei et al., 2002)
pcDNA3 Flag MKK7B2 Jnk2a2	Addgene: 19727 (Lei et al., 2002)
pCDNA3 Flag p38α	Addgene: 20351 (Enslen et al., 1998)
PCDNA3 Flag p38α (AGF)	Addgene: 20352 (Enslen et al., 1998)
pCDNA3 Flag MKK6 (Ala)	Addgene: 13519 (Raingeaud et al., 1996)
pCDNA3 Flag MKK6 (Glu)	Addgene: 13518 (Raingeaud et al., 1996)
pCDNA3 Flag MKK4	Addgene: 14615 (Derijard et al., 1995)
pENTR1A no ccDB (w48-1)	Addgene: 17398 (Campeau et al., 2009)
pLenti CMVtight Puro DEST (w768-1)	Addgene: 26430 (Campeau et al., 2009)
pLenti CMV rtTA3 Blast (w756-1)	Addgene: 26429 (Campeau et al., 2009)
pLenti CMVtight eGFP Puro (w771-1)	Addgene: 26431 (Campeau et al., 2009)
pLenti CRISPR v2	Addgene: 52961 (Sanjana et al., 2014)
pRc/RSV Flag MKK3 (Ala)	Addgene: 14670 (Raingeaud et al., 1996)
pRc/RSV Flag MKK3 (Glu)	Addgene: 14669 (Raingeaud et al., 1996)

BACTERIAL STRAINS

E.coli XL1-Blue MRF	Stratagene, La Jolla, US-CA
E. coli Stbl3	gift from Dr. Phil Cheng

EUKARYOTIC CELL LINES

3T3-J2	ATCC, Manassas, US-VA
COS-1	ATCC, Manassas, US-VA
KerTr	ATCC, Manassas, US-VA
THP-1	ATCC, Manassas, US-VA
HEK-293T	ATCC, Manassas, US-VA

STANDARD BUFFERS AND SOLUTIONS

AP buffer

TRIS/HCl (pH 9.5)	100 mM
NaCl	100 mM
$MgCl_2$	5 mM

CoIP buffer

NaCl	100 mM
EDTA	15 mM
Triton-X100	0.1% (w/v)
TRIS/HCl (pH 7.5)	50 mM

PBS

NaCl	140 mM
KCl	30 mM
Na_2HPO_4	6.5 mM
KH_2PO_4	1.5 mM

Adjusted to pH 7.4

PBST	
PBS	
Tween	0.1%(v/v)

TBS	
NaCl	150 mM
TRIS/HCl	10 mM

Adjusted to pH 8.0

BIOLOGICAL SAMPLES FROM HEALTHY DONORS

Healthy human skin samples were obtained from the surgical division of the Tagesklinik für Kinderchirurgie, Fällanden, Switzerland. All biological samples were collected with informed written consent upon approval of Local Ethical Committees, according to the Declaration of Helsinki.

2.2 CELL BIOLOGICAL METHODS

CULTIVATION AND MAINTENANCE OF EUKARYOTIC CELLS

Cells were grown in petri dishes or flasks as adherence or suspension cultures. They were incubated in a CO_2 incubator (37 °C, 95% relative humidity, 5% CO_2), cultivated in growth medium and propagated as specified in the following table:

Cell type	Growth medium		Propagation	Passages
	Basal medium	Supplements		
3T3-J2	DMEM	10% FCS, 1% A/A	Detaching and splitting 1/5 when confluency was reached	~P10-P25
COS-1	DMEM	10% FCS, 1% A/A	Detaching and splitting 1/10 when confluency was reached	~P10-P35
HEK-293T	RPMI 1640	10% FCS, 1% A/A, Sodium Pyruvate (1 mM), GlutaMAX (1:100)	Splitting 1/10-1/25 when confluency was reached	~P10-P35
KerTr	Keratinocyte-SFM	EGF, BPE (supplied by manufacturer), 1% A/A	Splitting 1/5 when confluency was reached	~P10-P35
THP-1 macrophages	RPMI 1640	10% FCS, 1% A/A, Sodium Pyruvate (1 mM), GlutaMAX (1:100)	Splitting 1/10-1/25 at a density of about 106 cells/ml with a medium change every third day	~P10-P35
Human primary keratinocytes	Keratinocyte-SFM	EGF, BPE (supplied by manufacturer)	Detaching and splitting 1/3-1/5 at a density of about 90%, medium change every second day	up to P5
Murine bone marrow-derived macrophages	RPMI 1640	10% FCS, 1% A/A, mM-CSF (1000 U/ml), NEAA, Sodium Pyruvate (1 mM), GlutaMAX (1:100)	Differentiation for 8 days, subsequent detaching and seeding without mM-CSF, no further propagation	-

To passage adherent cells, they were washed three times with PBS. To detach from the plastic surface, they were incubated 4-5 min with a suitable amount of trypsin (0.05% [w/v] in PBS) at 37 °C in the incubator. The trypsinization was stopped by resuspending the cells in fresh medium containing at least 10% FCS and subsequent seeding. For human primary keratinocytes trypsinization was stopped by resuspending the cells in fresh medium and addition of a volume of FCS corresponding to the amount of trypsin. The cells were then centrifuged (200g, 3 min, RT) and resuspended in fresh medium with subsequent seeding.

For long-term storage, cells were suspended, centrifuged (200g, 4 min, RT) and resuspended in growth medium containing 10% DMSO. They were slowly frozen at ~1 °C/min in cryotubes and stored in liquid nitrogen.

ESTABLISHMENT OF PRIMARY EUKARYOTIC CELL CULTURES

Primary human keratinocytes isolation

The isolation of primary keratinocytes from foreskin is adapted from a previous report (Rheinwald and Green, 1975). Biopsies were from different donors aged 4-11 years (provided by the Tagesklinik für Kinderchirurgie, Fällanden, Switzerland). Foreskins were disinfected twice in 70% ethanol, washed in PBS and the fat tissues removed using sterile tweezers and scissors. Then the biopsies were cut in small pieces (8-10 mm x 4 mm), incubated for 90 min in DMEM (1% A/A without FCS) and left overnight at 4 °C in dispase II 4U/ml in PBS, pH 7. Afterwards, the epidermis was separated from the dermis with tweezers. To favor the separation, the skin pieces were incubated for additional 30 min in dispase II solution at 37 °C. The epidermis was then incubated for 20 min at 37 °C in a trypsin 0.25% / EDTA 0.02% / PBS solution. The epidermis was disrupted by gentle up and down pipetting with DMEM 25% FCS, 1% A/A using 10-ml serological pipettes and the cells passed through a 100 µm nylon strainer (BD, Franklin Lakes, US-NJ). After centrifugation, cells were resuspended in complete keratinocytes medium without EGF and seeded on mitomycin c treated 3T3-J2 feeder cells at a ratio of 5:1 (for 10cm dishes: 2 $x10^6$ keratinocytes and 0.4 $x10^6$ feeders). The day after, medium was changed to complete keratinocytes medium with EGF. Cells were maintained on feeders until they reached 60-70% confluency, then they were transferred on freshly mitomycin c treated 3T3-J2 feeder cells.

Complete keratinocyte medium

DMEM	3 parts	375 ml
Ham's F-12	1 part	125 ml
FCS	~10%	50 ml
Insulin (5 mg/ml)	5.2 µg/ml	600 µl
Hydrocortisone (200 µg/ml)	0.42 µg/ml	1.2 ml
Adenine (1.2 mg/ml)	21 µg/ml	10 ml
apo-Transferrin (5 mg/ml)	5.2 µg/ml	600 µl
3,3',5-Triiodthyrosine (2 µM)	2.1 nM	600 µl
Choleratoxin (1 µM)	0.1 nM	60 µl
Gentamycin (50 mg/ml)	5.2 µg/ml	60 µl
EGF (40 µg/ml)	10.5 ng/ml	150 µl

3T3-J2 feeders were treated for 2 hrs with 10 µg/ml mitomycin c in DMEM (10% FCS, 1% A/A). After 3 washing with PBS, the cells were trypsinized and seed at the desired density. Keratinocytes were plated on top 2 hrs later.

Primary mouse bone marrow-derived macrophages (BMMs)

Adult mice at an age of 8-14 weeks were sacrificed by CO_2 inhalation and disinfected with 70% ethanol. Femur and tibia were cut with a scissor and flushed with 1 ml of RPMI 1640 medium. The cells were collected, washed once by centrifugation (200g, 5 min, RT), resuspended in 25 ml of supplemented RPMI 1640 medium (10% FCS, 1%A/A, 1 mM Sodium Pyruvate, 1% GlutaMAX, 1% NEAA, 1000 U/ml mM-CSF) and passed through a cell strainer (70 µm nylon, BD, Franklin Lakes, US-NJ). Subsequently, the cells were seeded in 10 cm dishes and differentiated for 7 days in supplemented RPMI 1640 medium. After differentiation, the cells were detached from the plastic surface by gentle scratching, washed once in PBS by centrifugation, counted and seeded in RPMI 1640 medium (10% FCS, 1% A/A, 1 mM Sodium Pyruvate, 1% GlutaMAX) at required densities for experiments.

TRANSIENT TRANSFECTION OF CELLS WITH PLASMIDS

Eukaryotic cells were transfected using the Lipofectamine 2000 (Invitrogen, Basel, Switzerland) or TransIT-X2 (Mirus Bio Lcc, Madison, US-WI) according to the manufacturers' instructions. For COS-1 and HEK-293T cells 0.1-4 µg plasmid DNA per well of a 6-well plate was diluted in OptiMEM and cells were grown in OptiMEM medium after transfection. For human primary keratinocytes 0.1-2 µg plasmid DNA was diluted in pure keratinocyte-SFM. The cells were transfected in keratinocyte-SFM containing EGF and BPE and the medium was changed 5 h after transfection.

siRNA TRANSFECTION

Cells were transfected with 21-mer duplexes siRNAs using INTERFERin (PolyPlus, Illkirch, France) according to the manufacturers' instructions. Concentration of siRNA was adjusted to 10 nM and 1 µl/ml INTERFERin was used. Final culture volume was 1.2 ml per well of a 12-well plate.

INDUCIBLE OVEREXPRESSION OF PROTEINS IN THP-1 CELLS USING A LENTIVIRAL SYSTEM

To generate THP-1, which overexpress a gene of interest in an inducible manner, we used the lentiviral system described by Campeau et al. (Campeau et al., 2009). DNA was cloned with the appropriate restriction enzymes from expression vectors (pcDNA3 Flag MKK7B2 Jnk1a1 and pcDNA3 Flag MKK7B2 Jnk2a2) into the pENTR1A no ccDB (w48-1) vector that had been linearized by restriction enzymes. For this purpose, the pENTR1A vector and the flanking restriction sites of the gene of interest were digested. The cleaved pENTR1A vector was treated with calf intestinal phosphatase (CIP)

followed by purification on a 1% agarose gel using the Macherey-Nagel gel extraction kit. DNA insert and vector were ligated by T4 DNA ligase. Competent Stbl3 bacteria were transformed, single colonies picked, and DNA isolated by miniprep. After sequencing, the gene of interest was subcloned into the lentiviral pLenti CMVtight Puro DEST (w768-1) using the Gateway LR Clonase II enzyme mix (Life Technologies, Carlsbad, US-CA) according to the manufacturers' instructions. Subsequently, lentivirus was produced, cells infected, and expression induced. See below for a detailed protocol.

LENTIVIRUS PRODUCTION

Lentivirus was produced by transfection of HEK-293T cells with a mix of the two packaging vectors (psPAX2 and pMD2.G) and the plasmid of interest. The cells were plated in 6-cm dishes at $1.4x10^6$ cells per dish in DMEM (10% FCS, no A/A) and transfected the day after seeding. For transfection, the medium was changed to 2.5 ml DMEM (10% FCS, no A/A). Plasmids were mixed gently in a molar ratio of 1:3:4 (psPAX3:pMD2.G:plasmid of interest) to a total of 8 µg using TransIT-X2 transfection reagent (Mirus Bio Lcc, Madison, US-WI) according manufacturers' instructions. 44-48 hours post transfection, the supernatant of the HEK-293T cell was collected and centrifuged at 200g for 4 min at 4 °C. The supernatants of this centrifugation step were distributed to Eppendorf tubes and centrifuged at 16000g for 4 hrs. The resulting supernatant was carefully removed without disturbing the virus pellet and leaving 500 µl of medium in the tube. The concentrated virus was directly added to the cells.

IRRADIATION OF CELLS WITH UV LIGHT

30 min after a medium change, cells were irradiated with the desired dose of UVB. For this purpose, the cover of the culture dish was removed and the cells were exposed to a UV source (UV802L, Waldmann, Villingen-Schwenningen, Germany). For a dose of 86.4 mJ/cm^2 the distance to the irradiation source was 5 cm and the irradiation time was 6 min 56 sec. Control cells were covered with aluminium foil and placed under the UV lamp.

STIMULATION OF PROTEIN SECRETION FROM CULTURED CELLS

Murine bone marrow-derive macrophages ($1.2\text{-}3.3 \times 10^5$ cells/cm^2) were grown in 24-well plates (ELISA) or 6-well plates (Western blot), stimulated with 100 ng/ml ultra-pure LPS (upLPS) for 16 hrs and subsequently with 150 µg/ml MSU or 5 µM nigericin for 6 hrs. Human keratinocytes were either transfected with siRNA, treated with inhibitors or vehicle (DMSO). 48 hrs after transfection, the medium was changed if not otherwise indicated and cells were irradiated with UVB at a dose of 86.4 mJ/cm^2 or treated with 5 µM nigericin. For treatment with inhibitor the medium was changed before addition of inhibitor; cells were irradiated 30 min later. Secreted proteins were collected after 6 hrs.
THP-1 cells were grown in 24-well plates (ELISA, 0.4×10^5 cells/well) or 6-well plates (Western blot, 4 x 105 cells/well) and differentiated with 25 ng/ml PMA for 3 days. After a medium change,

differentiated cells were stimulated with 100 ng/ml upLPS for 16 h and subsequently with 150 µg/ml MSU or 5 µM nigericin for 6 hrs.
Transfected COS-1 and HEK-293T cells were left untreated and secreted proteins were collected 16-30 hrs after transfection.

MEASUREMENT OF PROTEIN RELEASE FROM STIMULATED CELLS

After stimulation of cells, the supernatant was removed and centrifuged at 1500g for 3 min to remove cellular debris. Cells left in the wells were lysed with a 5% Triton X-100 solution in PBS and used to determine total LDH. For Western blot analysis of secreted proteins, cells were cultured in serum-free media (OptiMEM or KSFM) and proteins were precipitated with acetone (2.5 volumes of acetone). For ELISA and LDH measurements supernatants and lysates were diluted as desired. Each experiment was performed in triplicate. ELISA and LDH measurements were performed in 96-well plates. Cytokine levels were determined by human IL-1β ELISA kit (R&D Systems, Minneapolis, US-MN) and TMB substrate kit (Pierce, Rockford, US-IL). LDH activity was measured by the CytoTox 96 assay (Promega, Madison, US-WC). All assays were performed according to the manufacturers' instructions. ODs were determined with the Cytation3 plate reader (BioTek, Winooski, US-VT).

IN VITRO DIFFERENTIATION OF KERATINOCYTES

Keratinocyte were grown in normal K-SFM medium until they reached confluency. Then, the medium was replaced with differentiation medium and the cells were cultured for 7 days. To assess differentiation, cells were lysed in 1x SDS sample loading buffer and further analyzed by Western blot.

Differentiation medium (DM)	
KBM CD ($CaCl_2$ 0.15 mM)	500 ml
ethanolamine	0.1 mM
phosphoethanolamine	0.1 mM

2.3 MICROBIOLOGICAL METHODS

CULTIVATION AND STORAGE OF E. COLI STRAINS

E. coli cells were suspended in an appropriate amount of LB-medium and grown at 37 °C on a shaker (180-230 rpm) until the required optical density at 600 nm (OD_{600}) was reached.

LB-Medium	
Tryptone	1% (w/v)
Yeast extract	0.5% (w/v)
NaCl	1% (w/v)

If required, 100 µg/ml ampicillin was added to the medium. To cultivate *E. coli* on agar plates, they were spread over the plate and incubated at 37 °C. Agar plates were produced by adding 1.5% (w/v) agar to the LB-medium before autoclaving. This medium was poured into petri dishes. If required, an appropriate amount of antibiotic was added to the medium after cooling down to ~55 °C. Plates with *E.coli* were sealed with parafilm and stored at 4 °C.

PREPARATION OF TRANSFORMATION-COMPETENT E. COLI

A fresh overnight culture of transformation-competent *E. coli* (XL-1 Blue or Stbl3) was diluted 1:100 with LB-medium and shaken at 37 °C until the OD_{600} was between 0.4 and 0.5. After centrifugation (600g, 10 min, 4 °C), the bacterial pellet was carefully resuspended in cold, sterile 100 mM $MgCl_2$ solution (1/4 of the original volume). The mixture was incubated on ice for 30 min, centrifuged as described above and the pellet was resuspended in cold, sterile 100 mM $CaCl_2$ solution (1/50 of the original volume). The mixture was incubated 3-4 hrs on ice. Cold, sterile glycerol was added to a final concentration of 30%, the mixture was aliquoted and stored at -80 °C.

2.4 MOLECULAR BIOLOGICAL METHODS

TRANSFORMATION OF COMPETENT E. COLI WITH PLASMID DNA

100 µl transformation- competent E. coli cells were mixed with 10 µl plasmid or ligation mix (~100 ng DNA) and incubated on ice for 30-60 min. The mixture was subjected to a heat-shock (2 min at 42 °C) and put on ice. 600 µl LB-medium was added to the mix and shaken for 30 min at 37 °C. Subsequently, the mix was plated on LB-agar plates, containing the corresponding selection antibiotic, and incubated overnight at 37 °C.

PREPARATION OF PLASMID DNA FROM E. COLI

Small scale plasmid preparation with the Plasmid Midi Kit

Transformed bacteria were picked from an LB-agar plates and grew in LB medium for 8 hrs without exceeding a $OD_{600\ nm}$ of 0.8. Small bacterial cultures (max 6 ml) were then processed according to the manufacturers' instructions with the High Pure Plasmid Isolation kit (Roche, Rotkreuz, Switzerland) to obtain plasmid DNA. The obtained purity and amount of plasmid DNA is sufficient for a subsequent analysis by restriction enzyme digest or sequencing. Plasmids were stored at -20 °C.

Middle scale plasmid extraction with the Plasmid Midi/Maxi Kit

Middle scale plasmid production was obtained from 50 or 200 ml bacterial culture with the Genopure Plasmid Midi/Maxi kit (Roche, Rotkreuz, Switzerland). Plasmids were stored at -20 °C.

PREPARATION OF GENOMIC DNA FROM MOUSE EARS FOR GENOTYPING

DNA from mouse ear biopsies was isolated according to the HotSHOT genomic DNA preparation method (Truett et al., 2000). Mouse ears were clipped and 75 µl of alkaline lysis buffer were added to the biopsies. The samples were incubated in 1.5 ml Eppendorf tubes for 20 min at 95 °C. Afterwards, the tubes were cooled down to 4 °C and 75 µl of neutralization buffer were added. The supernatants were used for genotyping PCR.

Alkaline lysis reagent	
NaOH	25 mM
EDTA	0.2 mM

Neutralization reagent	
Tris/HCl	40 mM

DETERMINATION OF THE NUCLEIC ACID CONCENTRATION

In order to determine the concentration of DNA or RNA solutions, the absorption at 260 nm was measured. This was done using a NanoDrop ND-100 (NanoDrop, Wilmington, DE) according to the manufacturers' instructions. ddH_2O or the elution solution from the appropriate isolation kit was used as a blank. Quality and purity of nucleic acids was assessed by $OD_{260/280}$ and $OD_{260/230}$ ratios.

AGAROSE GEL ELECTROPHORESIS OF DNA

DNA molecules were separated by agarose gel electrophoresis for analytical and preparative purpose. Gels with an agarose content of 0.8-2% (w/v) in TAE buffer were used. Agarose concentration was dependent on the fragment length. Electrophoresis was performed at 100-150 V. To visualize DNA bands, GelGreen (Biotium, Hayward, US-CA) was diluted 1:10000 in the gel. The DNA samples were mixed with DNA Gel Loading Dye (Thermo Fisher Scientific, Waltham, US-MA) before loading them into the slots.

The bands were visualized with a blue LEDs DR-22A Transilluminator (Mobitec, Göttingen, Germany) and amber screen or photographed using Odyssey Fc (Li-cor, Lincoln, US-NE). DNA size standards were used to determine bands size. For cloning of fragments, the bands of interest were excised with a scalpel.

The DNA was purified from the gel with the gel extraction/PCR purification kit (Macherey-Nagel, Düren, Germany) according to the manufacturers' instructions.

POLYMERASE CHAIN REACTION (PCR)

PCR allows fast amplification of DNA fragments from a DNA template.

Preparative PCR reactions

Two primers flanking the coding sequence and containing introduced restriction sites complementary to those in the target expression vector were used. The following reaction mix was prepared in a PCR tube on ice:

PCR reaction mix	
Template DNA	~5-100 ng
5'-Primer (50-100 µM)	1 µl
3'-Primer (50-100 µM)	1 µl
dNTPs (2.5 mM each)	1 µl
Pfu DNA polymerase	0.5 µl
10 x PCR buffer	5 µl
ddH_2O	add. 50 µl

The PCR reaction was carried out in a Mastercycler gradient (Eppendorf, Hamburg, Germany) PCR machine. The temperature program was adjusted to the desired parameters - depending on fragment length, primer annealing temperature and specificity of the amplification.

PCR program			
First denaturing step	5-8 min	95 °C	
Denaturing step	1 min	94 °C	25-35 cycles
Primer annealing	30-60 sec	45-55 °C	
Primer extension	1-2 min	72 °C	
Last primer extension	10 min	72 °C	
Storage until analysis		4 °C	

Specificity and efficiency of the reaction were tested by running 5 µl of the reaction mix on an agarose gel. The reaction product was purified by preparative agarose gel electrophoresis with subsequent gel extraction.

DIGESTION OF DNA BY RESTRICTION ENZYMES

For an analytical digest 1 µg of plasmid DNA were digested with 5 U restriction endonuclease (in a volume of 10 µl. For preparative digest 10 µg of plasmid DNA was used. The appropriate buffer was used according to the recommendations of the manufacturer. For double digestion, each enzyme was used at the amount described above. The reaction tubes were incubated for 2 hrs at the recommended temperature. The digestion mix were used for gel electrophoresis and fragment purification.

DEPHOSPHORYLATION OF DNA ENDS

Alkaline phosphatase removes phosphate residues from DNA ends. Two ends without phosphate cannot be linked by *T4* DNA ligase. This can be used to avoid intra- and intermolecular ligation of vector DNA. Dephosphorylation was done mainly with vectors, which had been digested with a single

restriction enzyme or to avoid self-ligation after incomplete digestion with two enzymes. The reaction was carried out in a final volume of 50 µl with 0.5 µl calf intestinal phosphatase (CIP; 30 U/µl). Incubation time was 1 h at 37 °C.

DNA LIGATION

DNA insert and vector were ligated by *T4* DNA ligase. The optimal stoichiometric ratio of vector to insert was assumed to be ~1:4 for sticky end ligations and ~1:10 for blunt end ligations. The following reaction mix was incubated for 6-12 h at 4 °C or RT.

DNA ligation mix	
10 x Ligation buffer	2 µl
Vector	1 µl
Insert	4 µl/10 µl
T4 ligase (U/µl)	1 µl
ATP (100 M)	1 µl
ddH_2O	add. 20 ml

Before transformation of *E.coli*, ligase was inactivated for 10 min at 60 °C.

ISOLATION OF TOTAL CELLULAR RNA FROM EUKARYOTIC CELLS

RNA isolation was performed from one well of a 6-well plate using the High Pure RNA Isolation Kit (Roche, Rotkreuz, Switzerland) according to the manufacturers' instructions. RNA samples were store at -80 °C.

PREPARATION OF CDNA BY REVERSE TRANSCRIPTION

Synthesis of cDNA from total cellular RNA was performed using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, US-MA) according to the manufacturers' instructions using oligo dT primers.

QUANTITATIVE REAL-TIME PCR (QRT-PCR)

Relative quantification of gene expression at the RNA level was performed the LightCycler 480 Sybr Green Master (Roche, Rotkreuz, Switzerland). The real-time PCR was performed with cDNA from total cellular RNA and a primer pair designed to a fragment of ~150 bp in length flanking an intron-exon border of the desired gene. To quantify the relative expression level of a certain gene, two reaction mixes were prepared. One contained the primer pair targeting the gene of interest, the other contained a primer pair targeting the internal reference gene *RPL27*. Forward and revers primers were premixed

at a concentration of 3.75 µM each. The following mixture was prepared on ice in LightCycler 96 or 480 Multiwell detection plates:

qPCR reaction mix	
Template cDNA (diluted 1:10	1 µl
Primers mix (3.75 µM each)	0.5 µl
2 x Sybr Green PCR Master Mix	5 µl
ddH_2O	add. 10 µl

The PCR reaction and detection were performed in the LightCycler 480 (Roche, Rotkreuz, Switzerland) according to the manufacturers' instructions. The following temperature program was applied:

Real-time PCR program			
Initiation step	2 min	50 °C	
First denaturing step	10 min	95 °C	
Denaturing step	10 sec	95 °C	40 cycles
Primer annealing and extension	30 sec	58 °C	
	15 sec	95 °C	
	20 sec	60 °C	dissociation curve
	15 sec	95 °C	

Specificity of the reaction was ensured by surveying the dissociation curve of a given primer pair. Data processing was performed using the LightCycler 480 software provided by the manufacturers according to the guidelines. Data evaluation and statistical analysis followed the rules of the ΔΔCT method described by the system manufacturer.

CLONING OF SGRNA INTO LENTIVIRAL VECTOR

Single guide RNA (sgRNA) were designed using the online Benchling platform (https://benchling.com). Single-stranded DNA oligonucleotides obtained from Microsynth (Balgach, Switzerland) were cloned into the lentiCRISPRv2 plasmid (Addgene, #52961) as described by Sanjana *et al.* (Sanjana et al., 2014).

The following reaction mix was prepared to phosphorylate and anneal the ssDNA oligos:

Forward oligo	1 µl
Reverse oligo	1 µl
10x *T4* NEBuffer	1 µl
T4 PNK	1 µl
ddH_2O	add. 10 µl

The reaction was incubated for 30 min at 37 °C followed by inactivation at 95 °C for 5 min using a Mastercycler gradient (Eppendorf, Hamburg, Germany) PCR machine. The reaction was cooled down from 95 °C to 25 °C at a rate of 5 °C/min. The annealed oligos were then cloned into pLentiCRISPRv2, previously linearized with BsmBI restriction enzyme and dephosphorylated with CIP. The overnight ligation reaction (16 hrs at 4 °C) was prepared as follow

pLentiCRISPRv2 (BsmBI dig, CIPed)	50 ng
Phosphorylated annealed oligos (1:200 diluted)	1 µl
10x T4 ligase buffer	2 µl
T4 ligase	1 µl
ddH_2O	add. 20 µl

Stbl3 bacteria were transformed with 5-10 µl of ligated pLentiCRISPRv2 and plated on LB/ampicillin agar plates. Colonies were picked and small scale plasmid preparation was performed.

2.5 General protein methods

Determination of protein concentration

Protein concentration of a solution was determined by the BCA protein assay (Pierce, Rockford, US-IL). The assay was performed in duplicates according to the manufacturers' instructions using 96-well plates and a BSA serial dilution in the appropriate buffer for the standard curve. Optical densities were measured by the Cytation3 plate reader (BioTek, Winooski, US-VT).

Acetone precipitation

2.5 volumes of acetone were mixed with the sample. After overnight incubation at -20 °C the mixture was centrifuged (16000g, 30-60 min, 4 °C) and acetone was removed. The pellet was resuspended in 1x SDS sample loading buffer before complete drying.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

To separate proteins according to their size by SDS-PAGE, the buffer system of Laemmli (Laemmli, 1970) was used. This contains glycine as a zwitter ion. The electrophoresis was carried out in vertical direction using the Mini-PROTEAN gel system (Bio-Rad, Hercules, US-CA) in 1 mm thick polyacrylamide gels of appropriate acrylamide concentration depending on the protein size. The gel contains a 1 cm long 5% stacking gel and a 6 cm long 8-20% separating gel.
Before loading the samples, they were mixed with an equal volume of 2 x sample loading buffer and incubated for 5 min at 95 °C. Electrophoresis was performed at 75 V until the loading dye reached the separating gel and then at 100-140 V until the loading dye reached the bottom of the gel. Prestained protein molecular weight marker (Thermo Fisher Scientific, Waltham, US-MA) or Prestained Protein Ladder V (Geneaid, Taipei, Taiwan) were used in all gels.

Separating gel (4 mini gels or one large gel)

Acrylamide concentration	8%	10%	12.5%	15%	18%	20%
Acrylamide/bisacrylamide (30:0.8)	11.4 ml	13.3 ml	16.8 ml	20.0 ml	23 ml	24.2 ml
TRIS/HCl (1M, pH 8.8)	15.0 ml	15.0 ml	15.0 ml	15.0 ml	15.0 ml	15.0 ml
ddH2O	13.4 ml	11.5 ml	7.4 ml	4.2 ml	1.2 ml	-
SDS (10% [w/v])	400 µl	400 µl	400 µl	400 µl	400 µl	400 µl
APS (20% [w/v])	170 µl	170 µl	200 µl	200 µl	200 µl	200 µl
TEMED	16 µl	16 µl	16 µl	16 µl	16 µl	16 µl

Stacking gel (4 mini gels)

Acrylamide concentration	5%
Acrylamide/bisacrylamide (30:0.8)	2.7 ml
TRIS/HCl (1M, pH 6.8)	2.0 ml
ddH2O	10.8 ml
SDS (10% [w/v])	160 µl
APS (20% [w/v])	80 µl
TEMED	16 µl

SDS-PAGE running buffer

TRIS/HCl (pH 8.0)	25 mM
Glycine	192 mM
SDS	0.1% (w/v)

2x SDS sample loading buffer

TRIS/HCl (pH 8.0)	100 mM
Glycerol	20% (v/v)
SDS	10% (v/v)
Bromophenol blue	0.01% (w/v)
DTT (1M)	20% (v/v)

MOBILITY-SHIFTS ASSAY

In order to separate phosphorylated protein by SDS-PAGE, 10% acrylamide gels were supplemented with the chemical PhosTag (Wako, Tokyo, Japan), a novel phosphate-binding tag at neutral pH.

PhosTag concentration	100 µM	50 µM
Acrylamide/bisacrylamide (30:0.8)	1.6 ml	1.6 ml
TRIS/HCl (1M, pH 8.8)	1.2 ml	1.2 ml
ddH_2O	1.72 ml	1.81 ml
SDS (10% [w/v])	48 µl	48 µl
APS (20% [w/v])	69 µl	69 µl
PhosTag	96 µl	48 µl
$MnCl_2$ (10 mM)	96 µl	48 µl
TEMED	4 µl	4 µl

Before blotting, the gels where incubated twice for 20 min with 1 mM EDTA in running buffer and further washed once with running buffer only.

WESTERN BLOT

For the detection of specific proteins by Western blot, electrophoretically separated proteins were transferred onto a nitrocellulose membrane. After blocking of unspecific binding sites, the membrane was incubated with the primary and the corresponding secondary antibody. Secondary antibodies are coupled to an enzyme that catalyzes the detection reaction.

Protein transfer by semi-dry blotting

Three sheets of Whatman 3MM paper were soaked in anode buffer I and placed on the anode side of the blotting apparatus. Two sheets of Whatman paper were soaked in anode buffer II and placed on top of the first sheets. The nitrocellulose membrane was equilibrated in anode buffer II and subsequently placed onto the Whatman papers, followed by the gel, which was carefully pressed onto the membrane to avoid air bubbles in between. Finally, three sheets of Whatman paper soaked in cathode buffer were place on top of the gel. Blotting was performed in a semi-dry blotter Trans-Blot Turbo Transfer System (Bio-Rad, Hercules, US-CA) at an electric current up to 1.0 A or voltage to 25 V.

Transfer buffer (Towbin buffer)	
Methanol	20% (v/v)
Tris base, pH 8.3	25 mM
Glycine	192 mM

Incubation with antibodies and visualization of protein bands

Unspecific binding sites were blocked with 3% milk powder (Migros, Zürich, Switzerland) in PBST (blocking solution) for at least 30 min. The membranes were then incubated with the primary antibody in blocking solution overnight at 4 °C. After washing three times in PBST for 5 min, the membranes were incubated with the corresponding secondary antibody in blocking solution for 30 min at RT. Secondary antibodies were coupled to alkaline phosphatise (AP). After incubation, the membranes were washed three times for 15 min.

AP detection was performed using the NBT/BCIP substrate kit (Promega, Madison, US-WC). The membrane was washed once with AP buffer and incubated in AP buffer containing 16.5 µl BCIP and 33 µl NBT per 5 ml of buffer. Reaction was stopped with H_2O when desired band intensities were obtained.

Imaging of blots and stained gels

Blots, films and stained gels were scanned using scanned using a LiDE210 scanner (Canon Inc., Tokyo, Japan). Images were acquired with 600 dpi resolution without filters or other altered acquisition settings. Digital image processing was performed using the Photoshop software (various versions, Adobe Systems Software, San Jose, CA). Processing was restricted to cutting. When the observed bands in the original blots were too weak for meaningful digital and print reproduction, brightness and contrast adjustments were applied to the whole image until the scientific result reflected the one seen on the original blot. Band intensity was quantified using ImageJ software (NIH, Bethesda, MD).

CO-IMMUNOPRECIPITATION (CO-IP)

CoIP of endogenous proteins in human primary keratinocytes

Human primary keratinocytes were grown to 70-90% confluency in 10 cm dishes, left untreated or irradiated with 86.4 mJ/cm^2 of UVB. One dish was harvested in 150 µl of CoIP buffer containing protease and phosphatase inhibitor cocktails (Bimake, Houston, US-TX) and homogenized by sonication. The lysates were centrifuged (16000g, 20 min, 4 °C) and the supernatant was used for CoIP. Protein concentration was assessed by BCA assay and 2 mg of proteins were incubated with 10 µg of antibody (anti-ASC [AL177] or anti-phosphoASC [P-Tyr-1000]) or with a corresponding amount of an isotype control overnight at 4 °C on a rocker. After centrifugation (1600g, 10 min, 4 °C), the supernatants were mixed with 150 µl of protein A sepharose (Amersham, Uppsala, Sweden or GE Healthcare, USA) and incubated for 90 min at 4 °C on a rocker. The protein A sepharose beads were washed 4 times with 1 ml of CoIP buffer and dissolved in 70 µl of 2x SDS sample loading buffer and heated for 5 min at 95 °C. Samples were then applied on SDS-PAGE gels, followed by Western blot analysis. Whole cell lysate was loaded as an input control.

CoIP of overexpressed proteins in COS-1, HEK-293T cells and HPKs

COS-1 or HEK-293T cells in 6 cm dishes were cotransfected with plasmids expressing the desired proteins, which, if necessary, were fused to a HA, Myc, VSV or FLAG tags. 24 to 30 hrs after transfection cells were harvested in 400 µl CoIP buffer containing protease and phosphatase inhibitor cocktails (Bimake, Houston, US-TX) and lysed by short sonication. Lysate was cleared by centrifugation (16000g, 10 min, 4 °C). 100 µl of lysate per reaction were incubated with antibodies against the used epitope tag or against the overexpressed protein at appropriate dilution overnight at 4 °C. 50 µl of protein A sepharose slurry were added and incubated for 1 hr at 4 °C. Incubation with protein A sepharose only served as control. Beads were washed three times with 600 µl CoIP buffer by centrifugation (400g, 2 min, 4 °C), resuspended in 50 µl 2x SDS sample loading buffer and heated for 5 min at 95 °C. Samples were then applied on SDS-PAGE gels, followed by Western blot analysis. Whole cell lysate was loaded as an input control.

CROSSLINKAGE OF ASC OLIGOMERS

Cells were lysed in a Triton X-100-containing buffer (0.5% Triton X-100 in 50 mM Tris/HCl, pH 7.6) as previously described (Hara et al., 2013). After centrifugation at 6.000 g for 30 min at 4 °C, pellets (Triton X-100-insoluble fraction, TIF) were resuspended in Tris-buffered saline (TBS) and the crosslinking agent disuccinimidyl suberate (DSS, Thermo Fischer Scientific) was added at a final concentration of 2 mM. After incubation for 45 min at 37 °C, the reaction was quenched with 1 M Tris/HCl, pH 7.5 and the pellets resuspended in SDS sample buffer.

2.6 ANIMAL EXPERIMENTS

ANIMALS

Mice with targeted caspase-1 ablation were kindly provided by the group of PD Dr. Hans-Dietmar Beer (University Hospital Zurich). JNK1 and JNK2 knockout animals were obtained from Jackson Laboratories (Bar Harbor, US-ME). All animals were housed and fed according to federal guidelines. All experiments involving mice were approved by the local veterinary authorities.

GENOTYPING

Genotyping of knockout mice was performed by PCR using the KAPA2G Fast HotStart PCR Kit (Kapa Biosystems, Wilmington, US-MA) and anaylzed on agarose gel.

PCR reaction mix	
Template DNA	1 µl
Primer (10 µM)	1.25 µl each
$MgCl_2$ (100 mM)	1 µl
dTNPs (2 mM each)	2 µl
5 U/µl KAPA2G Fast HotStart DNA Polymerase	0.1 µl
5X KAPA2G Buffer A	5 µl
ddH_2O	add. 25 µl

The PCR reaction was carried out in a Mastercycler gradient (Eppendorf, Hamburg, Germany) PCR machine.

PCR program			
Initial denaturation	3 min	95 °C	
Denaturing	15 sec	95 °C	30 cycles
Annealing	15 sec	60 °C	
Extension	15 sec	72 °C	
Final extension	10 min	72 °C	
Storage until analysis		4 °C	

For JNK1 knockout mice the wild-type allele results in a 378 bp fragment and the mutant in a 260 bp fragment, wherease for JNK2 the wild-type band come at 375 bp and the mutant at 270 bp.

MSU PERITONITIS

Mice were injected intraperitoneally with 1 mg MSU crystal in 2 ml sterile PBS. After 3 hrs, mice ere sacrificed by CO_2 inhalation and peritoneal cavity were washed with 7 ml of PBS. The lavage fluids were centrifuged at 200g for 5 min and total number of infiltrating cells was counted with a Neubauer chamber. Cells were further characterized by flow cytometry as follows: neutrophils ($Ly6G^+$ / $7/4^+$), infiltrating macrophages ($CD19^-/CD11c^-/CD11b^+/F4/80^{mid}$).

2.7 MICROSCOPY

IMMUNOFLUORESCENCE OF ASC SPECKS

HPKs were seeded on circular 18 mm glass coverslips (Hecht-Assisten, Sondheim/Rhön, Germany) in 12-well chamber plates for experiments. Thereafter, cells were fixed for 30 min in 3% PFA / 2% sucrose solution, permeabilized for 2 min with 0.2% Triton X-100 in PBS and blocked for 1 hr in 1% bovine serum albumin (BSA Fraction V, GE Healthcare) in PBST. The primary antibody against ASC (N-15R) was diluted in blocking buffer and incubated with the samples for 1 hr at room temperature. The DyLight488-conjugated secondary antibody (ab96891) was diluted with DAPI in blocking buffer and applied for 1 hr at room temperature. Coverslips were mounted on glass slide using antifading ProLong Gold and cured for 48 hrs before imaging. Cells were analyzed by confocal microscopy (SP5, Leica), image names were anonymized and independently analyzed with Imaris software (Bitplane, Belfast, UK).

IMAGE ACQUISITION BY CONFOCAL MICROSCOPY

Digital images from stained cells were recorded at the desired magnification and resolution by a Leica SP5 confocal microscope (Leica Microsystems, Wetzlar, Germany). Image names were anonymized and independently analyzed with Imaris software (Bitplane, Belfast, UK).

IMAGE ACQUISITION BY LIGHT MICROSCOPY

Digital images from cells in culture were recorded at the desired magnification and resolution by an Vert.A1 microscope (Carl Zeiss, Oberkochen, Germany) equipped with an AxioCam ERc5s camera controlled by the Axio vision software.

2.8 FLOW CYTOMETRY

Cells were collected, washed with PBS and transferred to 96-well U-bottom plates and washed again 3 times with FACS buffer (2% FCS in PBS). The cells were incubated with flow cytometry labeled antibodies in FACS buffer for 45 min on ice. After 3 washes with FACS buffer cells were resuspened in FACS buffer and directly analyzed using a FACSCanto (BD, Franklin Lakes, US-NJ) with FACS DIVA software (BD, Franklin Lakes, US-NJ) or fixed with 2% PFA and store at 4 °C in the dark for later processing.

2.9 STATISTICAL ANALYSIS, DATA ACQUISITION AND EVALUATION

Statistical analysis was performed using the Prism Software (GraphPad Software, San Diego, CA). When multiple groups were compared to one control group, one-way ANOVA with posterior Dunnett's correction was performed. Different treatments were compared to control treatment by Student-t test. Differences were considered significant when: *P $\leq$ 0.05, **P $\leq$ 0.001, ***P $\leq$ 0.0001 and ****P $\leq$ 0.00001.

CHAPTER 3: RESULTS

3.1 THE ROLE OF P38 IN INFLAMMASOME ACTIVATION IN KERATINOCYTES

This section is adapted from: **Gabriele Fenini**, Serena Grossi, Samuel Gehrke, Takashi K. Satoh, Hans-Dietmar Beer, Emmanuel Contassot and Lars E. French, "The p38 mitogen-activated protein kinase critically regulates human keratinocyte inflammasome activation" Manuscript submitted to *Journal of Investigative Dermatology,* in revision.

It has been reported that inflammasomes are involved in skin homeostasis and inflammatory skin diseases (Contassot et al., 2012; Satoh et al., 2015). Although inflammasomes have been mainly characterized in hematopoietic immune cells such as macrophages and dendritic cells, inflammasomes are also expressed and functional in keratinocytes (Beer et al., 2014). Indeed, keratinocytes are immunologically active cells equipped with functional pattern recognition receptors including the NOD-like receptor (NLR) family that, in their function as essential components of the inflammasome, can sense and respond to various environmental stimuli. These include UVB (290-320nm) (Feldmeyer et al., 2007), microbes (Soong et al., 2012; Reinholz et al., 2013), bee venom (Dombrowski et al., 2012), viral double-stranded RNA (Dai et al., 2017), irritants such as chromium (Adam et al., 2017) and contact sensitizers (Watanabe et al., 2007), but also to endogenous stimuli such as cytosolic DNA (Dombrowski et al., 2011; Goblos et al., 2016) or serum amyloid A (Yu et al., 2015). Given the broad range of stimuli that can trigger inflammasome activation, and especially the NLRP3 inflammasome in myeloid cells, it is currently believed that a common mechanism triggered by diverse activators is involved. Several kinases such NEK7, PKC-δ and PKR, have been associated with the activation of the inflammasome (Neumann and Ruland, 2013). Recent studies also revealed that NLRP3 inflammasome activation is critically dependent on the Syk kinase and the mitogen-activated protein kinase (MAPK) JNK in murine myeloid cells (Hara et al., 2013; Lin et al., 2015) and in the human monocytic THP-1 cell line (Okada et al., 2014). The MAPKs ERK, JNK and p38 are well described kinases activated by numerous environmental stresses, inflammatory mediators and infections (Arthur and Ley, 2013). For instance, UV-related stress immediately activates p38 and JNK (Rosette and Karin, 1996; Pfundt et al., 2001), and p38 MAPK plays a key role in UVB-induced inflammation through a process involving cyclooxygenase-2 (Chen et al., 2001).

While the role of the JNK in inflammasome activation in hematopoietic immune cells has been documented (Hara et al., 2013; Okada et al., 2014), the mechanisms regulating inflammasome activation in human keratinocytes has never been investigated to date. Here, we show that the p38 MAPK is critically involved in inflammasome activation in human primary keratinocytes.

UVB-INDUCED IL-1β SECRETION BY HUMAN KERATINOCYTES IS BLOCKED BY P38 INHIBITORS

UVB irradiation triggers several intracellular signaling cascades including MAPK pathways (Muthusamy and Piva, 2013) and inflammasome activation in human epidermal keratinocytes (Feldmeyer et al., 2007). We analyzed the effect of a panel of selective MAPK inhibitors on inflammasome activation in human primary keratinocytes (HPKs thereafter). UVB irradiation induced the release of IL-1β into the supernatant of control vehicle (DMSO)-exposed cells and this was blocked, as expected, by the cell-permeant pan-caspase inhibitor carbobenzoxy-valyl-alanyl-aspartyl-[O-methyl]- fluoromethylketone (Z-VAD) (Figure 9a). All four tested p38 inhibitors, namely p38 MAP kinase inhibitor I (PI), SB203580 (SB), VX-702 (VX) and BIRB 796 (BIRB), as well as the JNK inhibitor SP600125 (SP) strongly suppressed IL-1β secretion by HPKs in a dose-dependent manner, whereas the inhibition of ERK with U0126 (U) had no effect unless used at high concentrations associated with toxicity (Figure 9a). The inhibition of Syk with piceatannol (Pic) also resulted in reduced IL-1β release in a dose-dependent manner.

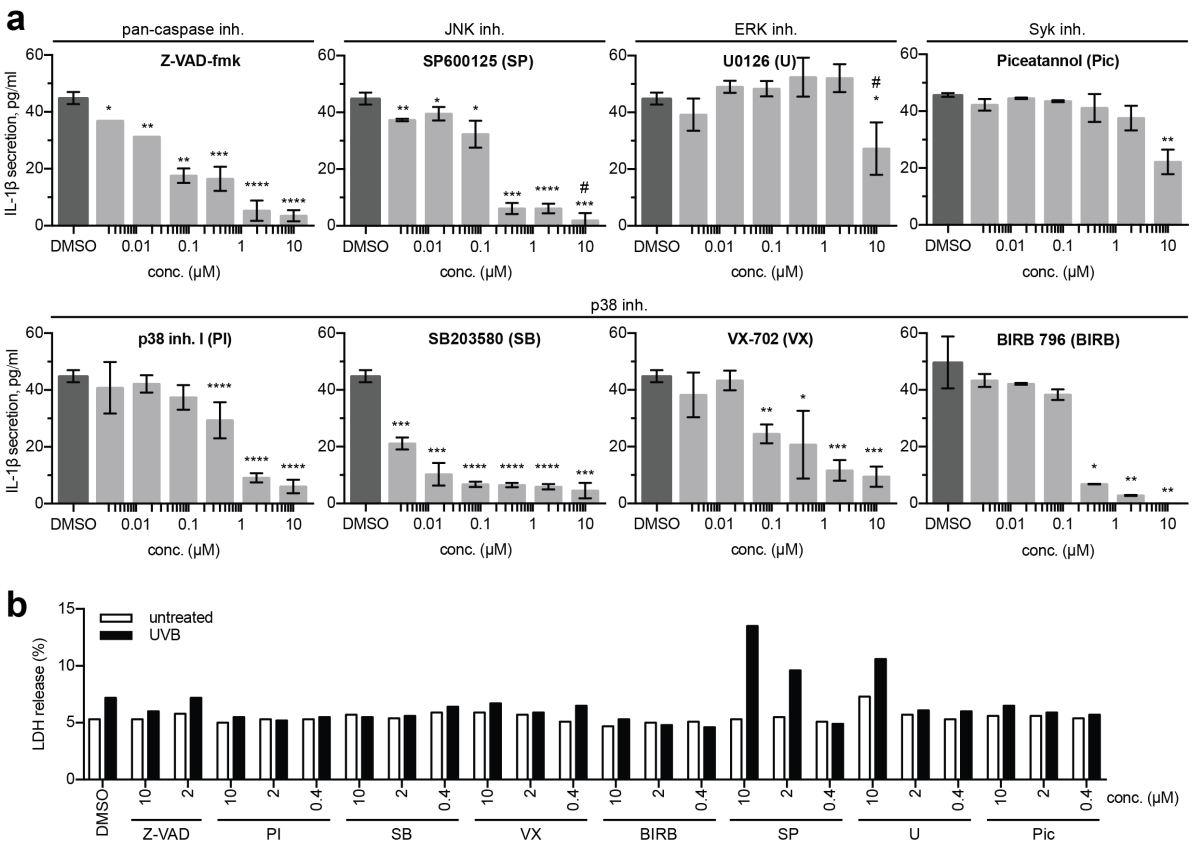

Figure 9 Pharmacological inhibition of p38, JNK and Syk impairs IL-1β secretion in human primary keratinocytes

(**a**) Human primary keratinocytes (HPKs) were exposed with the indicated inhibitors or vehicle (DMSO) at different concentrations and exposed to UVB 30 min later. (**b**) Cytotoxicity was measured by LDH release in HPKs exposed or not to UVB 30 min after inhibition treatment. Culture supernatants were collected 6 hrs after UVB irradiation and analyzed for LDH release or IL-1β secretion by ELISA. Data are expressed as the mean +/- SEM of three independent experiments with two-tailed unpaired t-test (**a**) or are representative of two independent experiments (**b**). # indicates elevated cytotoxicity as measured by LDH release (**b**).

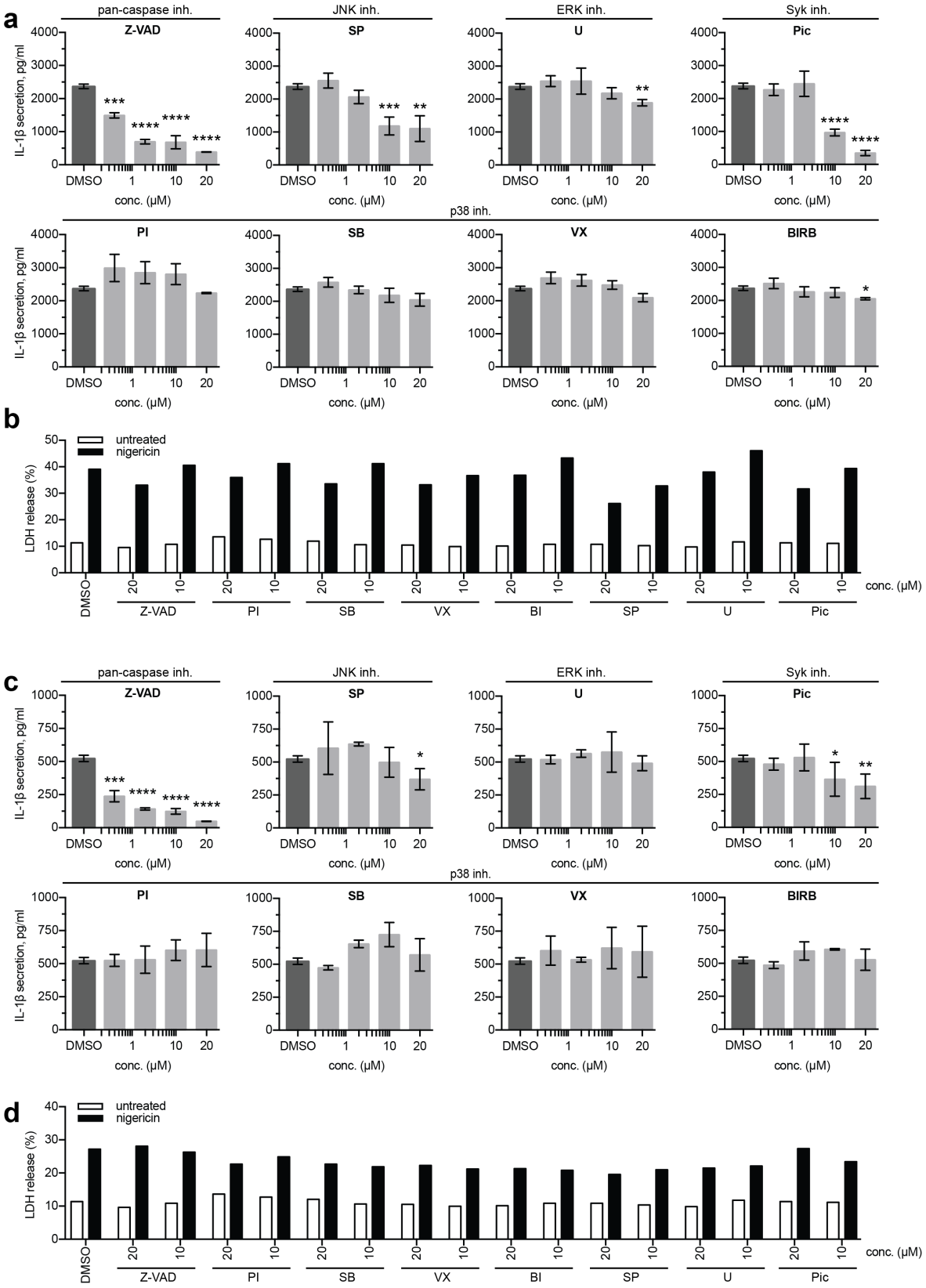

Figure 10 Pharmacological inhibition of JNK and Syk impairs IL-1β secretion in myeloid cells

(**a**) Human peripheral blood mononuclear cells (PBMCs) and (**b**) THP-1 cells were treated with the indicated inhibitors or vehicle (DMSO) at different concentrations and exposed to 5 μM nigericin 30 min later. Cytotoxicity was measured by LDH release in PBMCs (**c**) and THP-1 (**d**) exposed or not to nigericin 30 min after inhibitor treatment. Culture supernatants were collected 6 hrs after nigericin exposure and analyzed

for LDH release or IL-1β secretion by ELISA. Data are expressed as the mean +/- SD and are representative of two independent experiments (**a-d**) with two-tailed unpaired t-test (**a**, **c**).

In contrast, IL-1β secretion induced in peripheral blood mononuclear cells (PBMCs) and in the monocytic cell line THP-1 by the pore-forming toxin nigericin, a widely used inflammasome activator (Mariathasan et al., 2006), was unaffected by the tested p38 inhibitors whereas, as previously reported (Hara et al., 2013), inhibition of JNK and Syk resulted in reduced IL-1β secretion (Figure 10a, b and c, d).

Next, we determined how long before or after UVB irradiation MAPK inhibitors could block the secretion of IL-1β. p38 inhibitors could strongly suppress inflammasome activation when added to HPK cultures 30 min before or at the time of UVB irradiation as well as up to 45 min after UVB-irradiation (Figure 11a). The inhibitory effect became more modest after 1 hr and was lost when the inhibitor was added to cell cultures 2 hrs after UVB irradiation (Figure 11c). The JNK inhibitor SP was able to inhibit IL-1β secretion induced by UVB only when given 30 min before irradiation whereas the Sky inhibitor piceatannol showed strong inhibition up to 4 hrs after irradiation (Figure 11b and c).

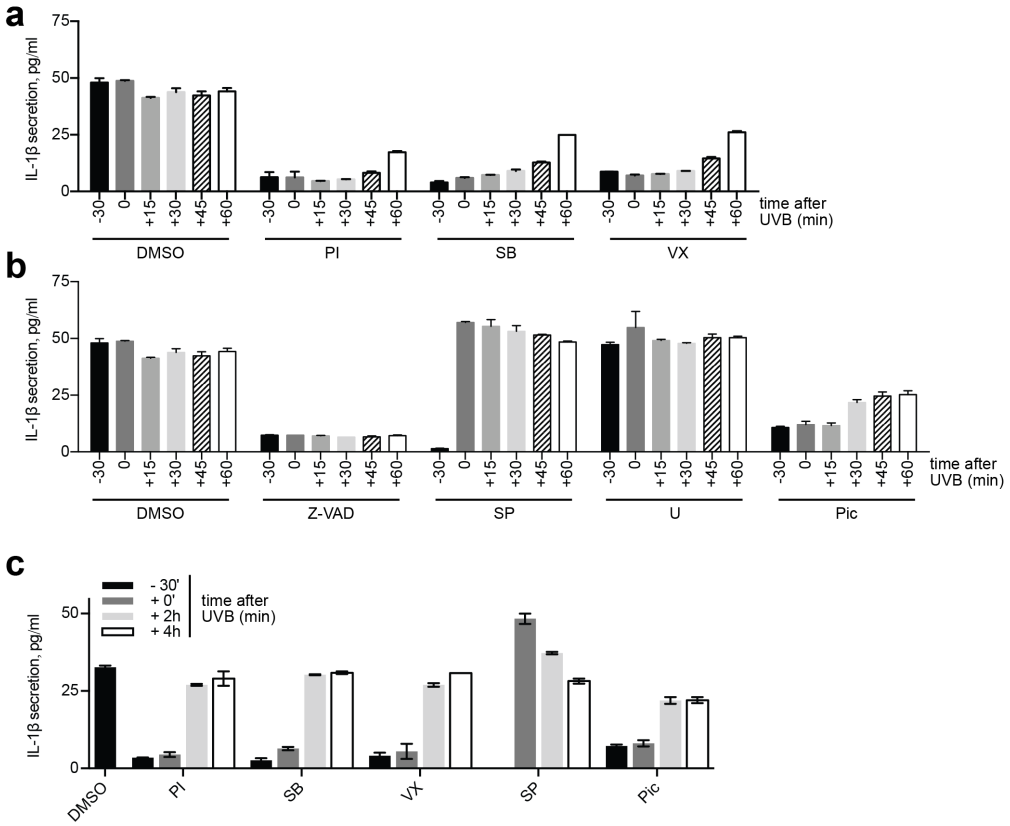

Figure 11 Inhibition of p38 blocks IL-1β release up to 45 minutes after UVB-irradiation

(**a-c**) Human primary keratinocytes (HPKs) were exposed to inhibitors of p38 MAP kinase I (PI; 2 μM), SB203580 (SB; 2 μM), VX-702 (VX; 2 μM) the pan-caspase inhibitor Z-VAD (5μM), the JNK inhibitor SP600125 (SP; 2 μM), the ERK inhibitor U0126 (U; 2 μM), the Syk inhibitor piceatannol (Pic; 5 μM) or vehicle (DMSO) 30 min prior to UVB irradiation, or at the indicated time points after irradiation after UVB irradiation. Culture supernatants were collected 6 hrs after UVB irradiation and IL-1β secretion was measured by ELISA. Data are expressed as the mean +/- and are representative of two independent experiments.

Keratinocytes, in contrast to myeloid cells, express IL-1β and IL-18 constitutively (Feldmeyer et al., 2007; Nestle et al., 2009). Macrophages and dendritic cells require a first stimulus, such as LPS, to induce the expression of inflammasome components, a process termed "priming". Since MAPKs are involved in the regulation of gene expression, we wondered if their pharmacological inhibition could impair IL-1β secretion by silencing the transcription of genes encoding individual inflammasome components (NLRs, ASC, caspase-1) or IL-1β. It has been described that HPKs can be stimulated by IFNγ and TNFα to strongly induce expression of inflammasome components (Strittmatter et al., 2016). We therefore primed the cells by exposing them overnight to IFNγ and TNFα. This resulted in an increased secretion of IL-1β upon UVB irradiation when compare to unprimed keratinocytes (Figure 12a). Exposure of the cells to the p38 inhibitor PI just before irradiation was able to inhibit IL-1β secretion. The combinatory treatment with IFNγ/TNFα showed a stronger effect but a spontaneous secretion in non-irradiated cells could be observed. We therefore decided to use IFNγ alone as priming agent and we analyzed by quantitative PCR its effect on the expression of inflammasome components. Interestingly, caspase-1 and NLRP3 showed a strong upregulation of 5.3- and 11.3-fold, respectively, whereas ASC and NLRP1 expression was not significantly increased (1.2-fold, p=0.05893 and 1.1-fold, p=0.15127) (Figure 12b). Moreover, IFNγ priming caused a 2-fold upregulation of IL-1β mRNA levels but had an opposite effect on the expression of IL-18 (0.7-fold, p=0.03234). Importantly, at the steady state, NLRP1 mRNA expression was more than 100 times higher than that of NLRP3 and after priming this difference was reduced of one order of magnitude. Next, we assessed the effect of p38 inhibition at the protein level. In both IFNγ-primed and -unprimed HPKs, the expression of caspase-1, ASC, NLRP1, NLRP3, pro-IL-1β and pro-IL-18 remained unaffected by the p38 inhibitors PI, SB and VX (Figure 12c). As shown also by qPCR, the levels of NLRP3 are very low in keratinocytes and the protein band is barely detectable even after IFNγ priming (black arrow in Figure 12c). In contrast, the secretion of IL-1β into the supernatant, as measured by ELISA (Figure 12d), was dramatically inhibited by p38 inhibitors in UVB-exposed HPKs irrespective of the priming with IFNγ. Furthermore, the three p38 inhibitors reduced the maturation of both IL-1β and IL-18 in UVB and nigericin-treated cells (Figure 12e). Similarly, also the JNK and Syk inhibitors strongly reduced IL-1β release in treated HPKs, whereas the inhibition of ERK had no effect (Figure 12f). These results show that, in our experimental settings, p38 is needed for inflammasome activation in HPKs and subsequent IL-1β maturation but not for the transcription of genes encoding inflammasome components, IL-1β or IL-18. JNK and Syk are also required for the activation of the inflammasome in keratinocytes as in other cell types such as macrophages and dendritic cells (Hara et al., 2013; Kistowska et al., 2014a; Okada et al., 2014; Lin et al., 2015) but since p38 inhibitors had no effect in myeloid cells, we decided to focus on the characterization of p38 in keratinocytes.

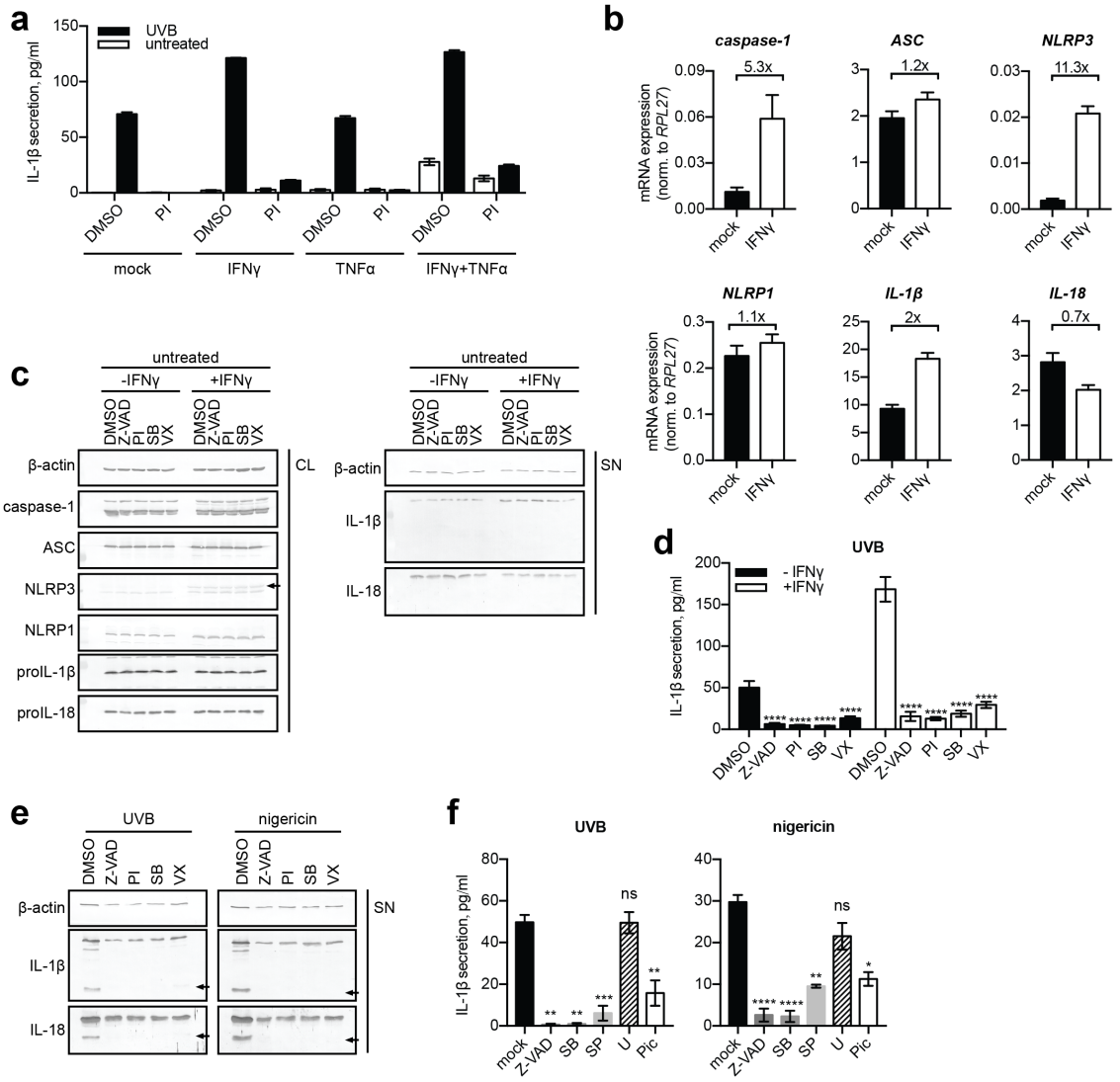

Figure 12 p38 inhibition impairs IL-1β secretion in UVB-irradiated human primary keratinocytes independently from its transcriptional activity

(**a**) Human primary keratinocytes (HPKs) were primed overnight with IFNγ (20 ng/ml), TNFα (10 ng/ml), or combined, then treated with the p38 inhibitor PI (2 μM) or vehicle (DMSO) and exposed to UVB 30 min later. Culture supernatants were collected 6 hrs after UVB irradiation and IL-1β secretion was measured by ELISA. (**b**) mRNA levels of the indicated genes (graph titles) in HPKs unprimed (mock) or treated overnight with IFNγ (20 ng/ml) were determined by qPCR; *RPL27* was used as housekeeper gene. Fold increases are annotated in the graphs. (**c**, **d**) HPKs were primed or not with 20 ng/ml IFNγ overnight and then exposed to the pan-caspase inhibitor Z-VAD (5μM) or the inhibitors of p38 MAP kinase I (PI; 2 μM), SB (2 μM) and VX-702 (VX; 2 μM) 30 min prior to UVB irradiation. After 6 hrs, cell lysates (CL) and supernatants (SN) were collected and the expression of the indicated proteins was analyzed by Western blot (**c**) and IL-1β secretion was measured by ELISA (**d**). (**e**) HPKs were exposed to Z-VAD (5 μM) or PI, SB and VX p38 inhibitors (all at 2 μM) 30 min prior to UVB irradiation or nigericin exposure. After 6 hrs, culture supernatants were collected and IL-1β and IL-18 secretion was measured by Western blot. (**f**) HPKs were treated with the pan-caspase inhibitor (Z-VAD; 5 μM), p38 (SB; 2 μM), JNK (SP; 2 μM), ERK (U; 2 μM) or Syk (piceatannol, Pic; 10 μM) or vehicle (DMSO) 30 min prior to UVB irradiation or nigericin exposure. After 6 hrs, culture supernatants were collected and IL-1β secretion was measured by ELISA. Data are expressed as the mean +/- SEM of at least three independent experiments (**d**, **f**) or are representative of two independent experiments (**a, b**; mean +/- SD of triplicates). One-way analysis of variance (ANOVA) with Dunnett's multiple-comparison test (**d**, **f**). Black arrows indicate NLPR3 (**c**) and mature IL-1β and IL-18 (**e**).

THE P38α AND δ ISOFORMS ARE INVOLVED INFLAMMASOME ACTIVATION IN KERATINOCYTES

Despite their high homology, p38α, β, γ and δ have different tissue expression, activators and downstream targets (Coulthard et al., 2009). In a first gene-silencing approach, we transfected HPKs with an siRNA library targeting, amongst others, the inflammasome components caspase-1, ASC, NLRP1, NLRP3 and AIM2 (Figure 13a) and each of the p38 isoforms, JNK1 and JNK2, ERK1 and ERK2 (Figure 13b). As expected, the knockdown of caspase-1 and ASC led to reduced IL-1β secretion after UVB or nigericin exposure. Interestingly, the knockdown of NLRP1 also led to a reduced IL-1β secretion, while the knockdown of NLRP3 and AIM2 had no impact. This observation is consistent with a previous report demonstrating that IL-1β secretion by keratinocytes exposed to UVB is also mediated by NLRP1 (Feldmeyer et al., 2007) but it also reveals that IL-1β secretion induced by nigericin, reported to be a NLRP3 activator in myeloid cells (Mariathasan et al., 2006), is impaired by the knockdown of NLRP1 in HPKs. Among MAPKs, the knockdown of JNK1 and p38α resulted in the strongest IL-1β inhibition, whereas the knockdown of ERK1 caused an increased secretion of IL-1β. Further investigation of the role of p38 in inflammasome activation was performed using larger-scale cultures of HPKs transfected with siRNAs to all individual p38 isoforms and to caspase-1 as positive control. p38α, β and δ isoforms were strongly expressed in keratinocytes, while p38γ was hardly detectably by Western blot. Importantly, knocking down p38 isoforms with siRNA did not affect the expression levels of pro-IL-1β or the inflammasome components caspase-1 and ASC (Figure 13c). As observed with the siRNA library, we not only confirmed the important role of p38α but repeatedly observed that p38δ is also involved in inflammasome activation upon UVB or nigericin exposure as revealed by reduced IL-1β and IL-18 secretion by HPKs transfected with siRNA to these two isoforms (Figure 13d, e and f). Although modest, we also observed a significantly decreased UVB-induced IL-18 secretion upon siRNA silencing of p38γ and the individual knockdown of all four p38 isoforms led to an important decrease of IL-18 release from HPKs exposed to nigericin. Altogether, siRNA silencing experiments show that p38α and p38δ are required for UVB- and nigericin-induced NLRP1-inflammasome-mediated IL-1β secretion in HPKs.

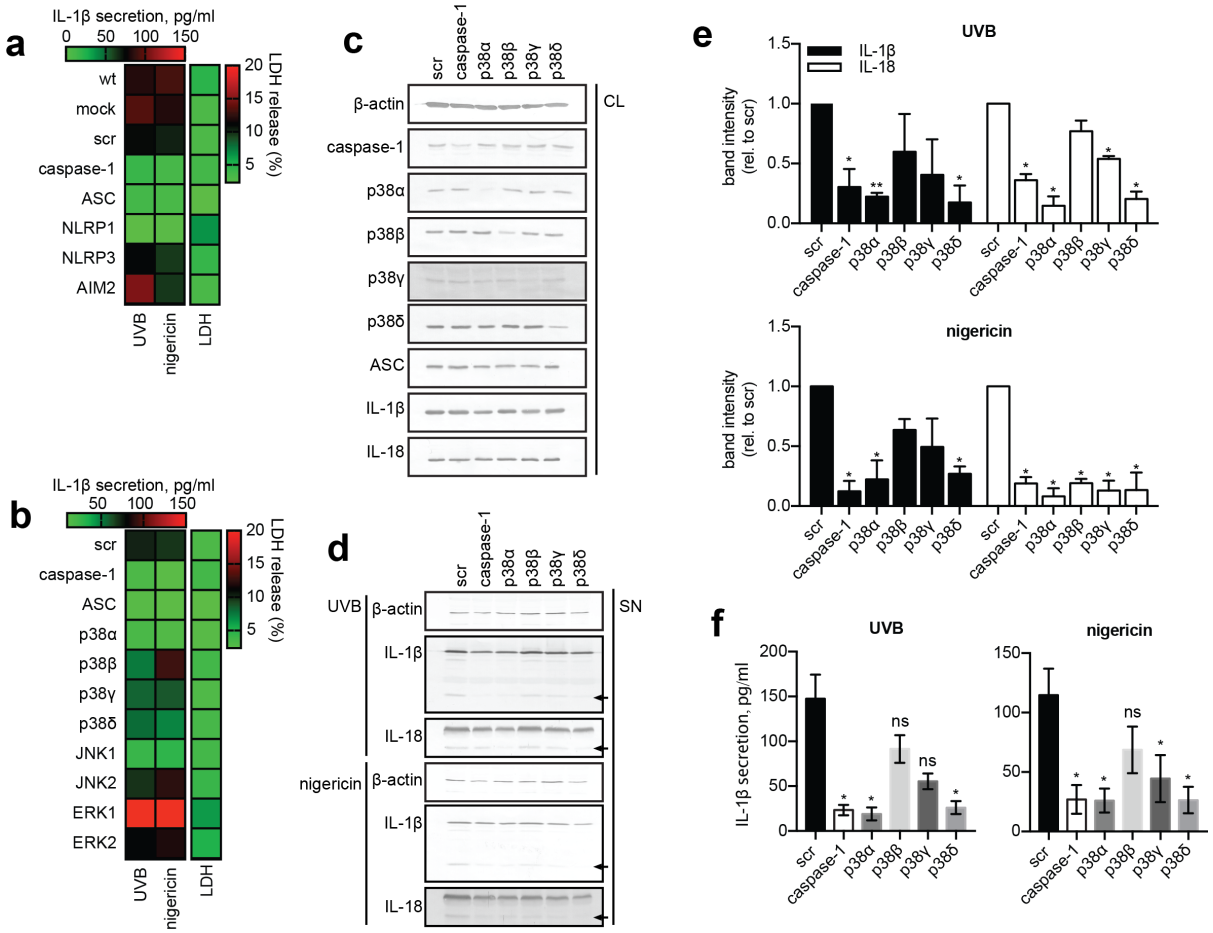

Figure 13 siRNA gene silencing of p38 isomers reveals a predominant role of p38α and p38δ in IL-1β secretion in human primary keratinocytes

(**a**, **b**) HPKs were transfected for 48 hrs with the indicated siRNA (10 nM) and irradiated with UVB or exposed to nigericin. IL-1β secretion was measured by ELISA 6 hrs later. Cell death was measured by LDH release. (**c-f**) HPKs were transfected for 48 hrs with siRNA (10 nM) to caspase-1 or to p38α, β, γ, and δ. The expression of the indicated proteins in non-stimulated cell lysates (CL) was analyzed by Western blot 48 hrs after transfection (**c**) and the release of IL-1β and IL-18 was analyzed in siRNA-transfected HPKs lysates 6 hrs after UVB irradiation or nigericin exposure by Western blot (**d**), band intensity quantification in (**e**) and ELISA (**f**). Data are representative of three independent experiments (**c**, **d**, **e**) and presented as mean +/- SD of duplicates with two-tailed unpaired t-test or expressed as the mean +/- SEM of seven independent experiments with one-way analysis of variance (ANOVA) with Dunnett's multiple-comparison test (**f**). Black arrows indicate mature IL-1β and IL-18 (**d**).

DELETION OF P38α BY CRISPR/CAS9 IMPAIRS INFLAMMASOME ACTIVATION IN KERATINOCYTES

Next, we used CRISPR/Cas9 technology to deplete p38 isoforms in HPKs, as well as ASC and caspase-1 as inflammasome silencing controls. To obtain stably depleted cells, we first used the KerTr keratinocyte cell line. As assessed by Western blot, the deletion of p38 isoforms, ASC and caspase-1 by CRISPR/Cas9 was optimal in the KerTr keratinocyte cell line and p38 deletion did not affect the expression levels of pro-IL-1β and the inflammasome components ASC and caspase-1 (Figure 14a). Consistently with the data obtained with siRNA silencing, the deletion of caspase-1, ASC and NLRP1

resulted in complete inhibition of IL-1β secretion by KerTr cells exposed to UVB (Figure 14b). Similarly, p38α- and p38δ-deletion in KerTr cells significantly reduced IL-1β secretion.

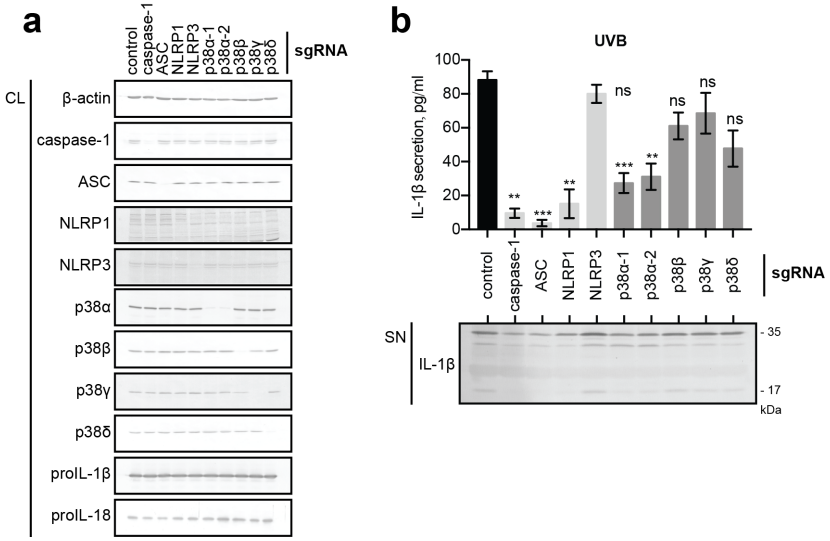

Figure 14 CRISPR/Cas9-driven knockout evidences a role of p38α and NLRP1 in UVB-mediated IL-1β secretion in KerTr cell line
(**a**) Caspase-1, NLRP1, NLRP3, ASC and p38 were depleted using CRISPR/Cas9 technology in KerTr cells. Expression of the indicated proteins in CRISPR/Cas9-targeted KerTr was analyzed by Western blot of the cell lysate (CL). (**b**) CRISPR/Cas9-targeted KerTr were irradiated with UVB and IL-1β secretion was measured 6 hrs after by ELISA and Western blot. The depleted proteins are indicated on the X-axis. Data are expressed as the mean +/- SEM of three independent experiments with one-way analysis of variance (ANOVA) with Dunnett's multiple-comparison test (**b**) or are representative of three independent experiments (**a**).

Our results showing a predominant role of NLRP1 in UVB-irradiated and nigericin-treated keratinocytes (Figure 13a, Figure 14b) were unexpected, since both stimuli have been reported to be activators of the NLRP3 inflammasome (Mariathasan et al., 2006; Feldmeyer et al., 2007). We therefore decided to specifically block NLRP3 using its inhibitor MCC950, a small-molecule able to block canonical and non-canonical NLRP3-induced ASC oligomerization without interfering with NLRC4 and AIM2 activity or Toll-like receptor signaling (Coll et al., 2015). We incubated HPKs with different concentrations of MCC950 or with the pan-caspase inhibitor Z-VAD for 30 min before UVB irradiation or nigericin exposure (Figure 15a). NLRP3 inhibition with MCC950 partially impaired IL-β secretion in UVB irradiated and nigericin-treated cells exposed to a concentration ranging between 1 and 10 μM, consistent with the reported inhibitory concentration (Coll et al., 2015). In contrast, Z-VAD strongly inhibited the maturation of IL-1β. Interestingly, we could not observe any dose-dependent inhibitory effect of MCC950. Since NLRP3 expression level in keratinocytes is very low (Figure 12b), we enhanced its expression by priming the cells with IFNγ (Figure 15b). None of the MCC950 doses tested on primed keratinocytes showed an effect on IL-1β secretion. As control of NLRP3 inhibition, we also exposed PBMCs to the MCC950 inhibitor prior to MSU or nigericin exposure (Figure 15c). MCC950 successfully blocked the activation of the NLRP3 inflammasome upon exposure to both stimuli. These results further support our observations that NLRP3 has a minor role in UVB- and nigericin-induced IL-1β processing in HPKs, since its inhibition only marginally affects the secretion of this cytokine.

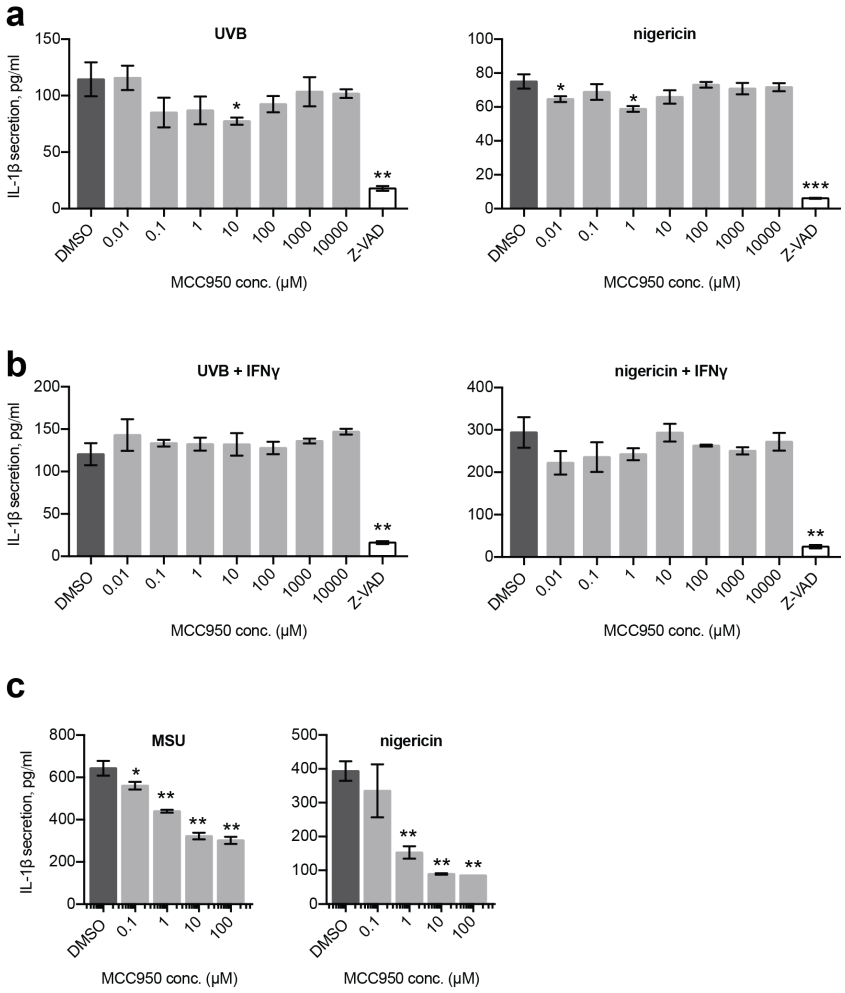

Figure 15 Specific inhibition of NLRP3 with MCC950 marginally impairs IL-1β secretion in human primary keratinocytes

(**a**) Human primary keratinocytes (HPKs) or (**b**) overnight IFNγ-primed (20 ng/ml) HPKs were incubated with the NLRP3 inhibitor MCC950 at the indicated concentrations, with pan-caspase inhibitor Z-VAD (5 μM) or with vehicle (DMSO) and exposed to UVB or nigericin 30 min later. (**c**) PBMCs were incubated with the NLRP3 inhibitor MCC950 at the indicated concentrations or with vehicle (DMSO) and exposed to MSU or nigericin 30 min later. Culture supernatants were collected 6 hrs after stimulation and IL-1β secretion was measured by ELISA. Data are expressed as the mean +/- SD and are representative of two independent experiments and analyzed with two-tailed unpaired t-test (**a**-**c**).

We then decided to apply the CRISPR/Cas9 technology to primary keratinocytes (see chapter 3.3 for the establishment of the method). Since deletion of p38α led to the strongest IL-1β inhibition in KerTr cells, we subsequently also deleted this isoform in HPKs using the CRISPR/Cas9 system. Optimal deletion of p38α using 2 different targeting sequences, as well as ASC and caspase-1, by CRISPR/Cas9 could also be achieved in HPKs and as previously observed, the deletion of p38α did not affect the expression levels of pro-IL-1β, pro-IL-18, ASC and caspase-1 (Figure 16a). While the deletion of ASC and caspase-1 led, as expected, to the abrogation of IL-1β and IL-18 release by HPKs exposed to UVB or nigericin, the deletion of p38α also resulted in a significant reduction in the mature form of IL-1β (p17) and IL-18 (p18) as revealed by Western blot analysis (black arrows in Figure 16b, band intensity

quantification in Figure 16c) and IL-1β secretion as revealed in culture supernatants by ELISA (Figure d).

Taken together, these results further support the key role of p38 MAPK, in particular the p38α isoform and, to a lesser extent, p38δ in NLRP1-inflammasome activation in HPKs.

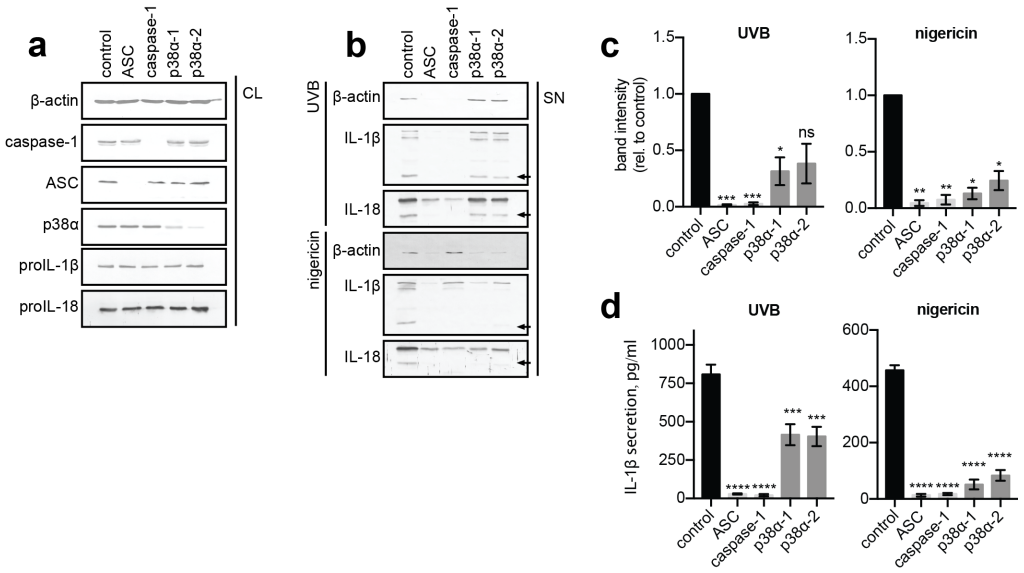

Figure 16 Deletion of p38 by CRISPR/Cas9 establishes key role of p38α in inflammasome activation in primary keratinocytes

(**a**) Caspase-1, ASC and p38α (2 sequences) were deleted using CRISPR/Cas9 technology in HPKs. Expression of the indicated proteins in CRISPR/Cas9-targeted HPKs was analyzed by Western blot. (**b**-**d**) CRISPR/Cas9-targeted HPKs were irradiated with UVB or exposed to nigericin and IL-1β secretion in the supernatant (SN) was analyzed 6 hrs after by Western blot (**b**), band intensity quantification in (**c**) and ELISA (**d**). IL-18 secretion was analyzed 6 hrs after stimulation by Western blot (**b**) and band intensity quantification in (**c**). Deleted proteins are indicated on the X-axis. Data are expressed as the mean +/- SEM of at least three independent experiments with one-way analysis of variance (ANOVA) with Dunnett's multiple-comparison test (**c**, **d**) or are representative of three independent experiments (**a**, **b**). Black arrows indicate mature IL-1β and IL-18 (**b**).

P38 INHIBITION PREVENTS ASC OLIGOMERIZATION IN KERATINOCYTES

ASC aggregates are composed of dimerized and oligomerized ASC. Such an oligomerization is required for the activation of caspase-1 and subsequent IL-1β maturation (Baroja-Mazo et al., 2014). ASC has been reported to form Triton X-100-resistant aggregates after inflammasome activation (Masumoto et al., 1999; Hara et al., 2013). Therefore, we prepared Triton X-soluble and -insoluble fractions from HPKs to assess the effect of p38 inhibition on ASC aggregation as another readout of inflammasome activity. Endogenous ASC was almost undetectable in the Triton X-insoluble fraction of HPKs but significantly increased 3 to 6 hrs after UVB irradiation (Figure 17a and b). The increased levels of ASC observed in Triton X-insoluble fractions of UVB- or nigericin-treated HPKs was diminished by the SB and VX p38 inhibitors and almost abolished by the inhibitor PI (Figure 17c). Of note, ASC was abundantly found in the Triton X-insoluble fractions from Z-VAD-treated cells irrespective of the stimulus (Figure 17c), demonstrating that blockade of the formation of ASC aggregates by p38 inhibitors is upstream of caspase-1 activation. Knockdown of p38α and p38δ by siRNA resulted in a

reduction of ASC oligomers in Triton X-insoluble fraction (Figure 17d), consistent with the IL-1β secretion pattern (Figure 13d-f). ASC oligomerization in UVB- or nigericin-treated keratinocytes was substantially decreased in p38α-CRISPR/Cas9-depleted HPKs (Figure 17e).

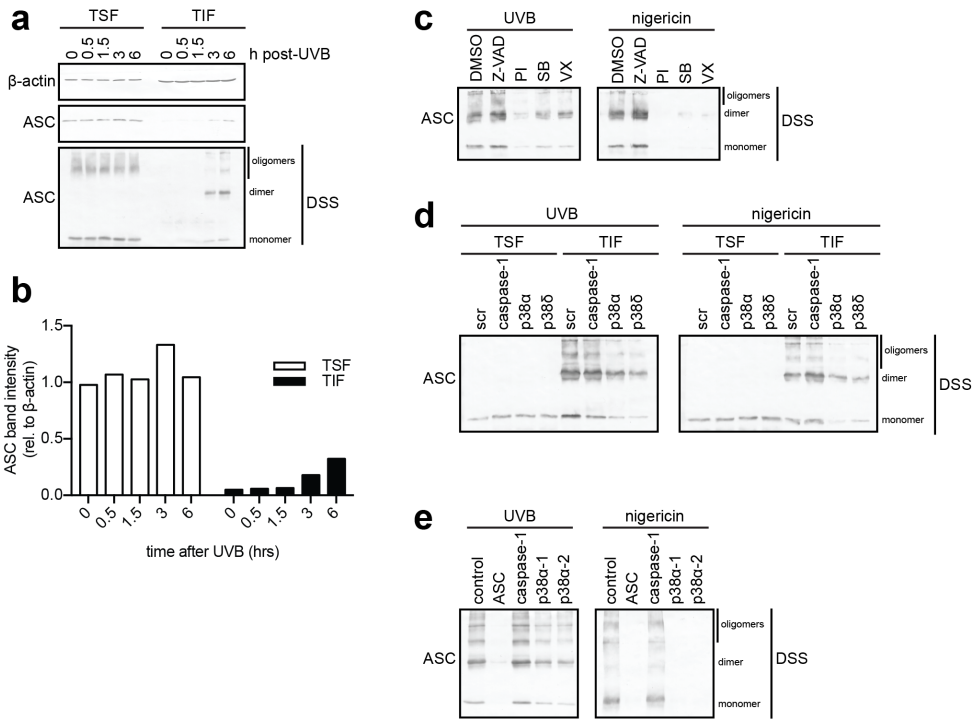

Figure 17 p38 is required for ASC oligomerization

(**a**) Immunoblot analysis of ASC and DSS-crosslinked ASC in the Triton X-100-soluble fraction (TSF) and -insoluble fraction (TIF) of HPKs 0.5, 1.5, 3 and 6 hrs after UVB irradiation. (**b**) Quantification of ASC bands intensity in TSF and TIF normalized to β-actin levels. (**c**) Immunoblot analysis of DSS-crosslinked ASC in the Triton X-100-insoluble fraction of UVB-irradiated HPKs exposed to Z-VAD or the p38 inhibitors PI (2 μM), SB203580 (SB; 2μM) and VX-702 (VX; 2μM) 30 min before. (**d**) Immunoblot analysis of DSS-crosslinked ASC in the Triton X-100-soluble fraction (TSF) and Triton X-100-insoluble fraction (TIF) of UVB-irradiated and nigericin-exposed HPKs transfected with siRNA to caspase-1, p38α and p38δ. (**e**) Immunoblot analysis of DSS-crosslinked ASC in the Triton X-100-insoluble fraction of UVB-irradiated and nigericin-exposed HPKs in which ASC or caspase-1 or p38α (2 sequences) were CRISPR/Cas9-deleted.

ASC oligomers, known as ASC specks, that can also be visualized microscopically, have been validated for the identification of cells in which the inflammasome is active (Hara et al., 2013; Satoh et al., 2013; Sahillioglu et al., 2014). All p38 inhibitors tested herein significantly decreased the number of ASC specks observed after UVB irradiation in HPKs (Figure 18a, b and Figure 19). Silencing of p38α and p38δ with siRNA in HPK (Figure 18c and d), as well as the deletion of p38α in HPK using CRISPR/Cas9 (Figure 18e and f) also resulted in a significant decrease in the number of UVB-induced ASC specks. Altogether, these results strongly suggest that, in HPKs, p38α and p38δ are critically involved in ASC oligomerization, a prerequisite for inflammasome activation.

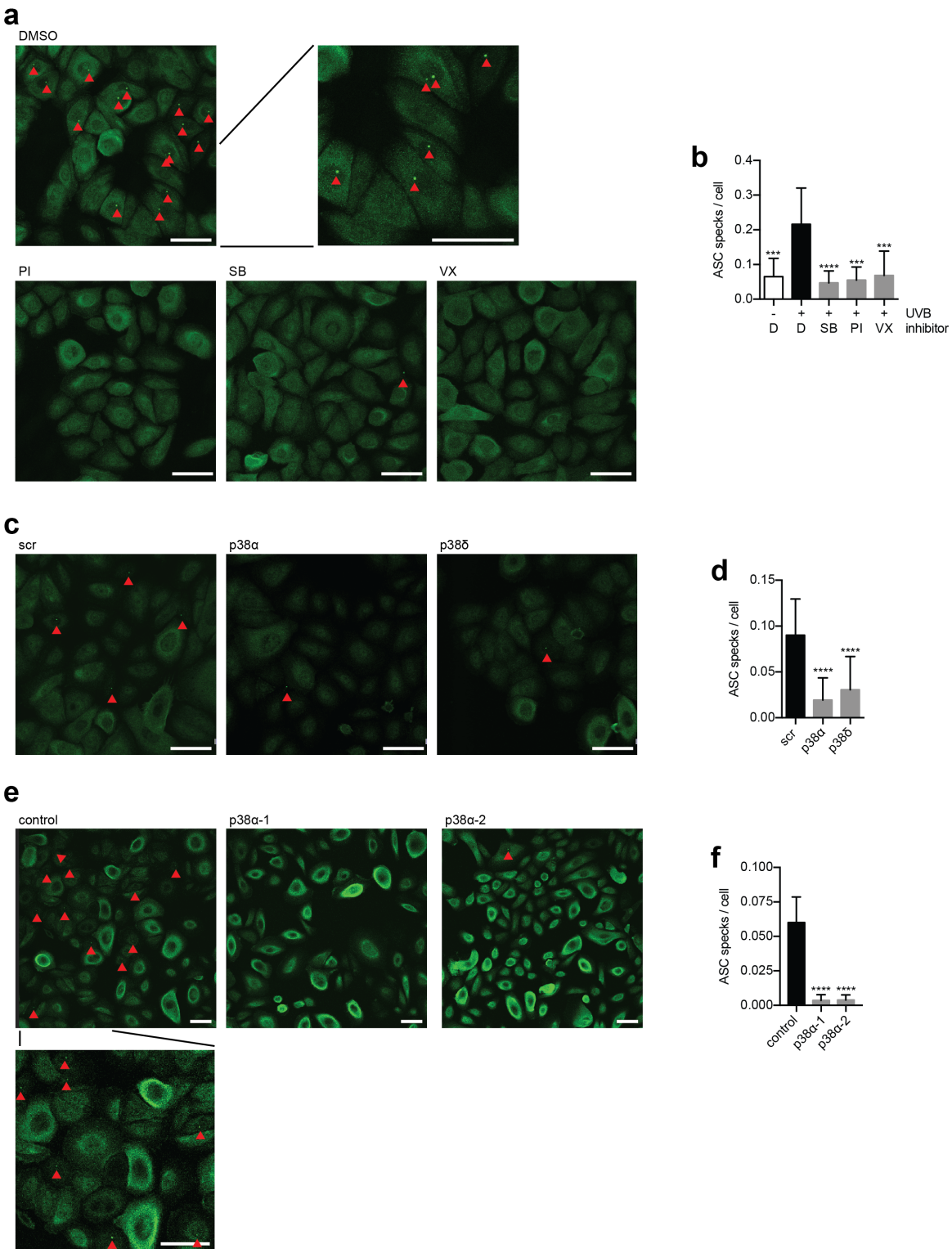

Figure 18 p38 is required for the formation of ASC specks

(**a**, **b**) Pictures and quantification of immunofluorescence analyses using an antibody to ASC of UVB-irradiated HPKs pre-treated with the p38 MAP kinase inhibitors PI (PI; 2 µM), SB203580 (SB; 2 µM) and VX-702 (VX; 2 µM) or (**c**, **d**) transfected with siRNA to p38α and p38δ. (**e**, **f**) Pictures and quantification of immunofluorescence analyses using an antibody to ASC of UVB-irradiated HPKs in which p38α (2 sequences) were deleted with CRISPR/Cas9. Green, ASC; red arrows indicate ASC specks. Scale bars, 50 µm. Data are representative of three (**b**, **d**) or two independent experiments (**f**), and are presented as mean +/- SD. Quantification of ASC specks (**b**, **d**, **f**) and statistical analysis (one-way ANOVA with Dunnett's multiple-comparison test).

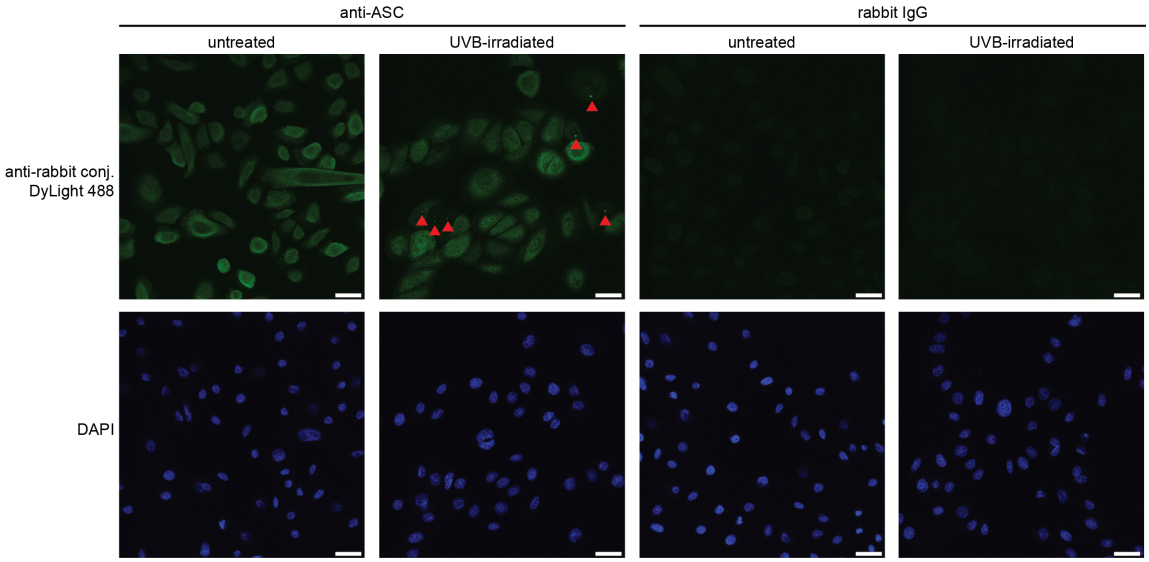

Figure 19 Specificity of the ASC antibody for the detection of specks

Immunofluorescence pictures of untreated and UVB-irradiated HPKs using an antibody to ASC or an IgG isotype control of rabbit origin. Green, ASC; red arrows indicate ASC specks. Scale bar, 50 µm.

ASC PHOSPHORYLATION IN HUMAN KERATINOCYTES

Several groups reported that phosphorylation of the adaptor protein ASC favors its oligomerization (Hara et al., 2013; Okada et al., 2014; Lin et al., 2015). They described that JNK and Syk kinases are involved in the phosphorylation of murine ASC on tyrosine 144 residue, corresponding to human Tyr146. We therefore addressed the question whether ASC was also phosphorylated in human primary keratinocytes and if p38 was involved in this process.

In a first approach, we immunoprecipitated (IP) endogenous ASC from untreated and UVB irradiated HPKs at different time points (Figure 20a). We then analyzed by Western blot the IP samples but we were unable to detect any phospho-tyrosine band at the expected ASC size of 24 kDa. Alternatively, we enriched samples by immunoprecipitating phospho-tyrosine (pTyr) containing proteins and blotting against ASC without detecting any substantial difference between untreated and irradiated samples (Figure 20b). Since the amount of phosphorylated ASC (pASC) could be only a minimal portion of the total protein, we decided to overexpress it by transfecting HPKs with a plasmid containing myc-tagged ASC and vesicular stomatitis virus (VSV)-tagged ASC as IP control. Twenty-four hours after transfection, we irradiated transfected HPKs with UVB or exposed them to nigericin (Figure 20c) or exposed to MAPK inhibitors prior to irradiation (Figure 20d). Next we performed an IP using an anti-myc antibody and checked for pTyr bands in the myc-IP fractions. In both experiments we failed to identify specific bands corresponding to pASC in primary keratinocytes stimulated with UVB and nigericin. Taken together, these results give a first indication that ASC may not be phosphorylated in HPKs upon inflammasome activation.

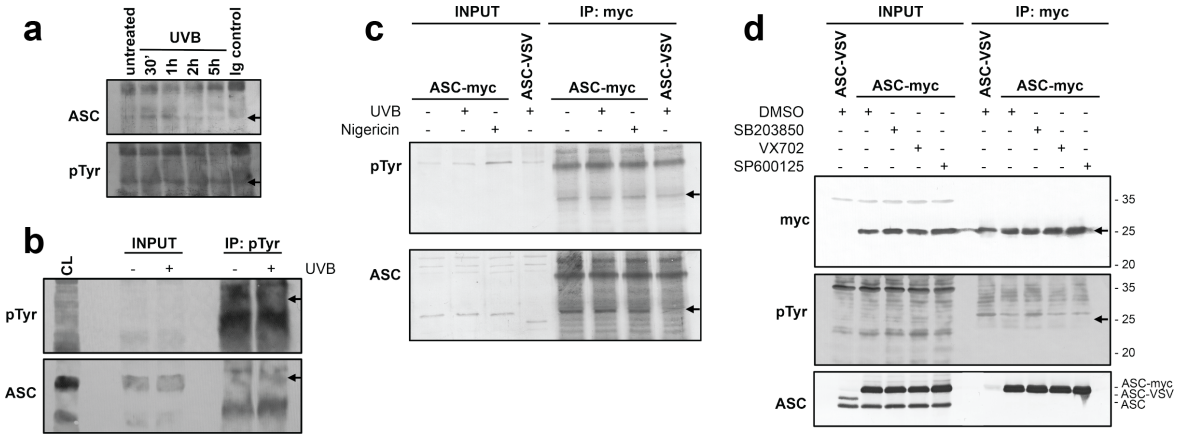

Figure 20 Identification of tyrosine-phosphorylated ASC in human primary keratinocytes

(**a**) Immunoprecipitation (IP) of endogenous ASC from lysate of human primary keratinocytes irradiated with 86.4 mJ/cm^2 UVB using an anti-ASC antibody and an anti-IgG antibody as control. (**b**) IP of phosphorylated tyrosine (pTyr) proteins from lysate of HPKs irradiated with UVB using an anti-pTyr antibody. (**c**, **d**) IP of overexpressed myc-tagged ASC (ASC-myc) from lysate of HPKs using an anti-myc antibody. Cells were transfected with expressing plasmids for 24 hrs and irradiated with UVB (**c**, **d**) or treated with 5 µM nigericin (**c**). HPKs were exposed 30 min before stimulation with DMSO, the p38 inhibitors SB203850 and VX-702 or the JNK inhibitor SP600125 at a concentration of 2 µM (**d**). Antibodies used for IP and Western blot are indicated. Black arrows indicate the expected size of ASC and ASC-myc.

To investigate if p38 is involved in the phosphorylation of ASC, we decided to co-overexpress human influenza hemagglutinin (HA)- and myc-tagged ASC in the presence of active p38α in the fibroblasts-like COS-1 cell line. These cells can be easily transfected with several constructs and have been extensively used to study inflammasome component interactions (Shoham et al., 2003; Munding et al., 2006). COS-1 were transfected with vectors expressing the wild-type p38α isoform or an inactive protein (p38α AGF), where both threonine and tyrosine in the conserved Thr-Gly-Tyr motif were mutated to alanine and phenylalanine, respectively (TGY→AGF)(Enslen et al., 1998). Cells were also transfected with a plasmid encoding MKK6, which was mutated on serine 207 and threonine 211 to glutamic acid (MKK6E) to mimic phosphorylation and thus activate the kinase (Raingeaud et al., 1996). 48 hrs after transfection cell lysates were incubated with anti-HA or anti-myc antibodies and IP samples analyzed by Western blot. pTyr antibody failed to detect any ASC-HA band in the IP samples but detected activated p38α (marked with * in Figure 21a). Also in the second experiment, it was not possible to detect ASC-HA or ASC-myc in the pTyr blot (Figure 21b). These results suggest that activated p38 is not able to induce the phosphorylation of ASC tyrosine residues.

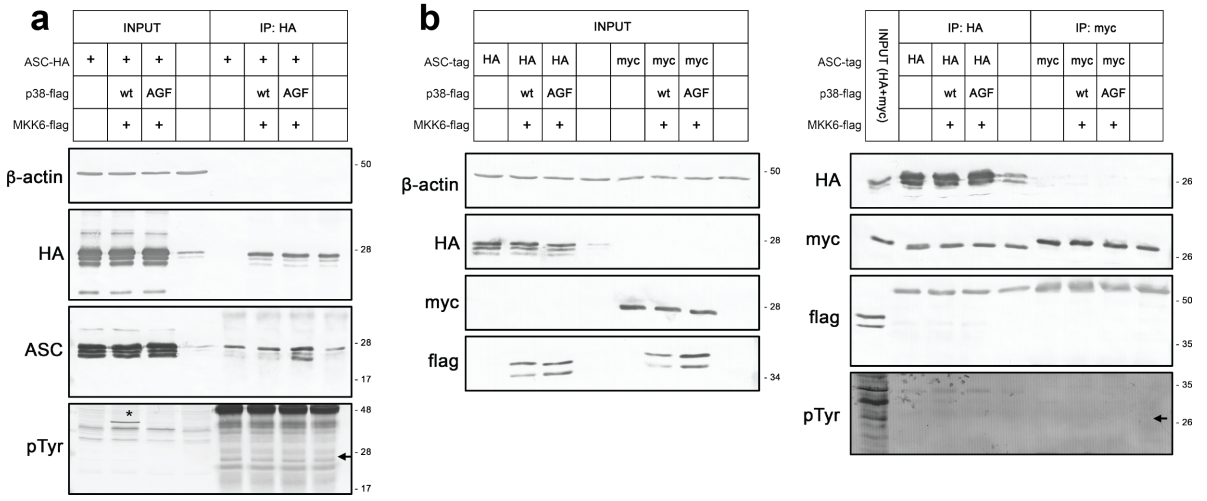

Figure 21 Overexpression in COS-1 cells of active p38 did not result in ASC phosphorylation on tyrosine residues

(**a**) Immunoprecipitation (IP) of overexpressed HA-tagged ASC (ASC-HA) using an anti-HA antibody from lysate of COS-1 cells co-transfected with wild-type (wt) or inactive (AGF) flag-tagged p38α (p38-flag) and active flag-tagged MKK6G (MKK6-flag) as indicated in the table. (**b**) IP of overexpressed ASC-HA or myc-tagged ASC (ASC-myc) using anti-HA or anti-myc antibodies from lysate of COS-1 cells co-transfected with wt or AGF p38-flag and MKK6-flag as indicated in the table. As control, lysates from mock-transfected cells were incubated with protein A sepharose. Antibodies used for IP and Western blot are indicated. * indicates p-p38-flag (wt) (**a**); black arrows indicate the expected size of ASC-HA and ASC-myc.

Since we could not detect any tyrosine phosphorylation of ASC by immunoprecipitation, we decided to try another approach, namely a mobility-shift assay. In this technique, SDS-PAGE gels are supplemented with PhosTag, a phosphate-binding tag, and phosphorylated proteins migrate slower and, when detected by Western blotting, are shifted on the blot (Kinoshita et al., 2009). We first analyzed p38 phosphorylation in HPKs upon UVB irradiation (Figure 22a). After 10 min, shifted p38 bands could be detected on the PhosTag(+) blot and these bands corresponded to phosphorylated p38 as confirmed with a p-p38 antibody. We next analyzed the lysate from irradiated keratinocytes for the detection of pASC (Figure 22b) but no detectable shift was observed. This could be due to the relatively low amount of pASC in comparison to the total amount of protein. Therefore, we transfected COS-1 cells with ASC-HA together with flag-tagged wild-type p38, active MKK6E and active MKK3E, analogously mutated on Ser189 and Thr193 to Glu (Raingeaud et al., 1996) (Figure 22c). As additional control, we transfected plasmid expressing active JNK1 and JNK2 (flag-MKK7-JNK1/2) (Lei et al., 2002). Analysis by Western blot of PhosTag(+) gels revealed phosphorylation of flag-p38 when expressed with both MKKs but again, we could not detect a shift of ASC-HA bands, also when expressed in combination with active JNKs. When activated, ASC form oligomers which are insoluble in Triton X-100-containing solution (Masumoto et al., 1999; Hara et al., 2013). We already showed that, upon UVB irradiation of primary keratinocytes, ASC levels in the Triton X-100 insoluble fraction (TIF) are augmented (Figure 17a and b). We therefore decided to analyze the TIFs from UVB-irradiated (Figure 22c) and nigericin-treated (Figure 22d) HPKs by PhosTag mobility-shift assay. Upon treatment, we could observe a time-dependent increase of ASC levels in the TIF which correlates with the decrease

seen in the soluble fraction (TSF). PhosTag(+) gels revealed a minor shift for all bands in the TIF. This was unlikely due to phosphorylation but rather from sample preparation. Overall, these experiments showed that phosphorylation of human ASC is hardly detectable and that, even though p38 clearly plays a role in the regulation of ASC oligomerization, this process seems to be independent from its inability to induce phosphorylation of ASC.

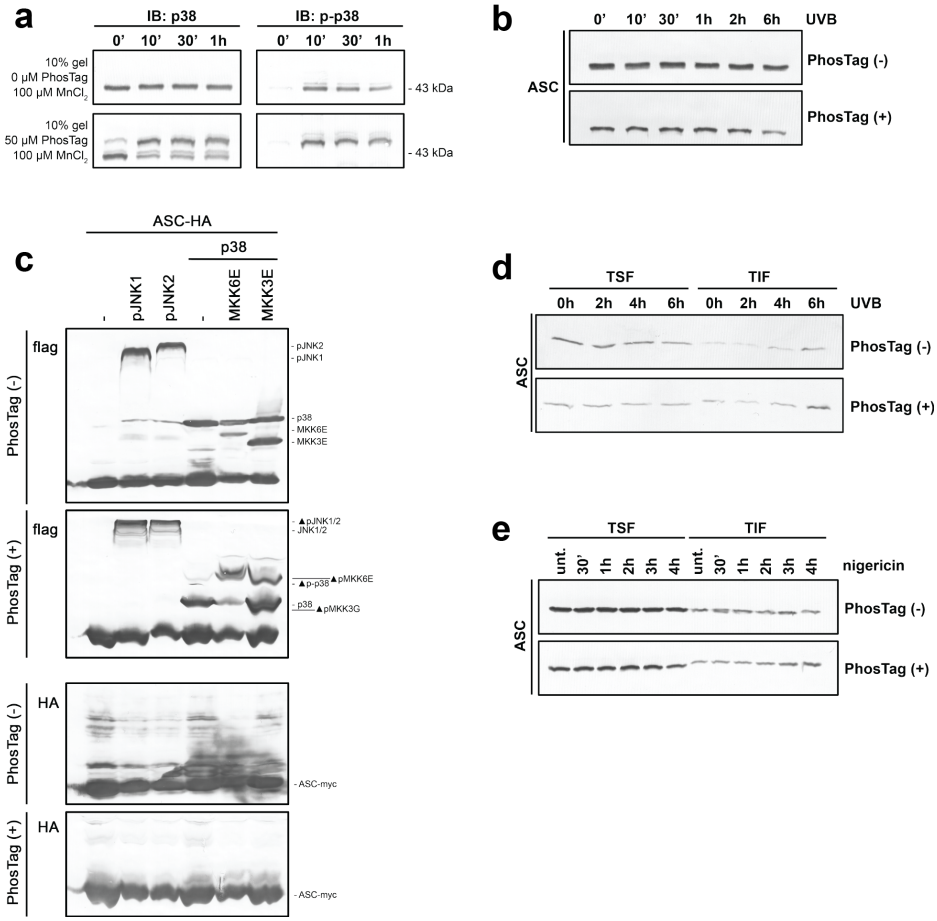

Figure 22 Identification of phosphorylated ASC by PhosTag mobility-shift assay

(**a**-**e**) Mobility-shift assay (MSA) with PhosTag supplemented SDS-PAGE gels (PhostTag(+)) or normal gel, (PhosTag(-)), as control. HPKs were left untreated, irradiated with with 86.4 mJ/cm^2 UVB (**a**, **b**, **d**) or 5 µM nigericin (**e**). Cell lysates were analyzed by MSA and blotted against p38 and p-p38 (**a**) or ASC (**b**). Triton X-100 soluble (TSF) and insoluble (TIF) were analyzed by MSA and blotted against ASC (**d**, **e**). (**c**) Immunoprecipitation of ASC-HA from lysates of COS-1 cells transfected for 48 hrs with constructs overexpressing ASC-HA and flag-tagged wild-type p38α (p38), active MKKs (MKK6E and MKK3E) and active pJNK1 and pJNK2; IP samples were analyzed by MSA and blotted against flag and HA. ▲indicates PhosTag shifted protein.

OVEREXPRESSION OF ACTIVE P38 IN HUMAN PRIMARY KERATINOCYTES RESULTS IN SPONTANEOUS IL-1β SECRETION

Pharmacological inhibition, knockdown and deletion of p38 in human primary keratinocytes impairs the activation of the inflammasome (Figure 9, Figure 13 and Figure 16). To confirm these observations with another approach, we overexpressed p38 alone or in combination with its MKKs in keratinocytes.

Overexpression of wild-type p38α did not result in spontaneous IL-1β secretion whereas, when co-expressed with active MKK6, mature p17 IL-1β was detected by Western blot (Figure 23a and c) and ELISA (Figure 23b and d). This was not due to increased expression of proIL-1β, since its levels in the lysates were similar in all tested conditions (Figure 23a and c). Co-expression of p38α with active MKK3 (MKK3E) or the inactive forms MKK6A and MKK3A, where both serine and threonine residues were mutated to alanine, did not cause spontaneous maturation of IL-1β. Also, both active or inactive MKKs transfected alone did not induce spontaneous maturation of IL-1β (Figure 23c). Interestingly, p-p38 was detected in all samples suggesting that the transfection itself could induce a stress response and subsequent MAPK pathway activation. None of the transfected construct affected IL-1β secretion upon UVB irradiation (Figure 23e). Altogether, these results further support the role of p38 in inflammasome activation since its inhibition blocked IL-1β maturation whereas, activation by overexpression of active MKK6 resulted in increased active IL-1β secretion.

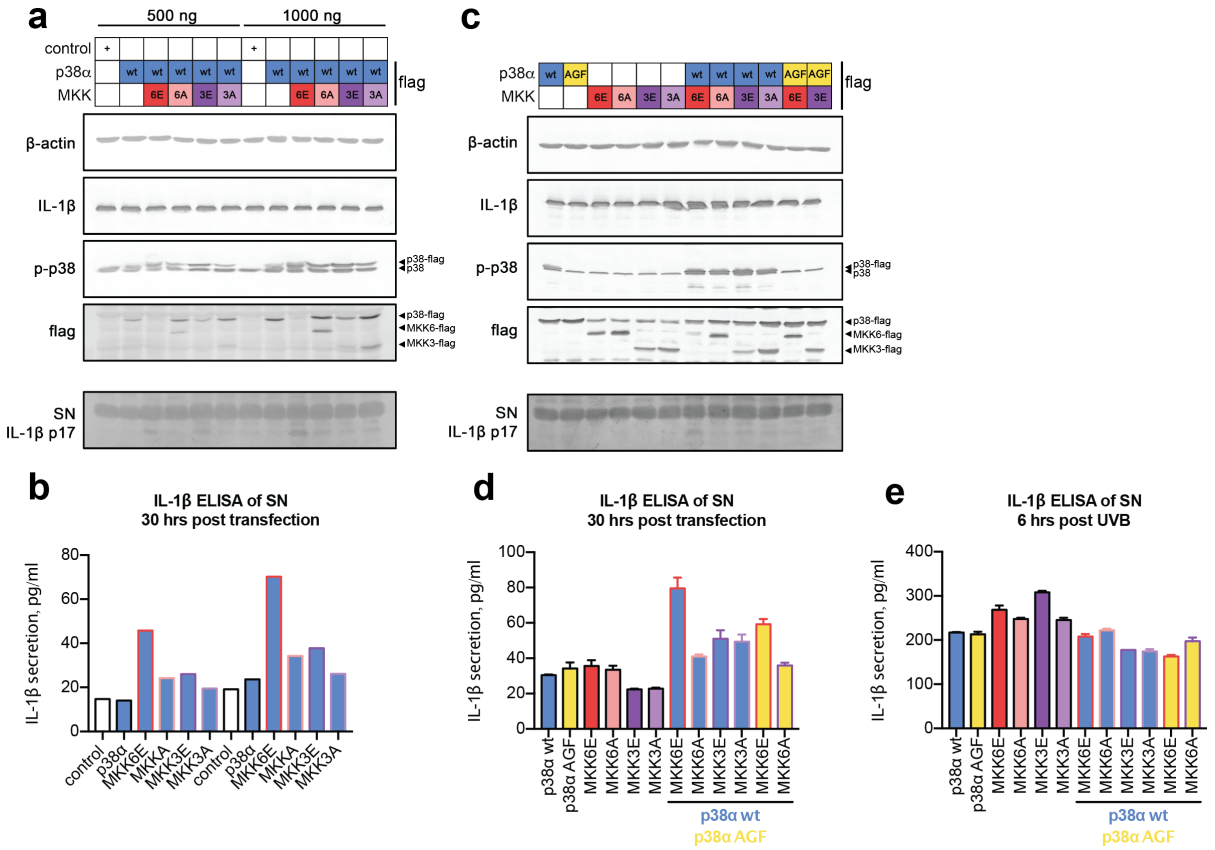

Figure 23 Active MKK6 overexpression with p38 in HPKs drives spontaneous IL-1β secretion

(**a**, **b**) HPKs were transfected for 30 hrs with 500 or 1000 ng of plasmids expressing wild-type (wt) or inactive (AGF) p38α, active MKK6E or MKK3E (6E, 3E) or inactive MKK6A or MKK3A (6A, 3A), as indicated in the table (**a**); as control, an empty vector was transfected. (**c**-**e**) HPKs were transfected for 30 hrs with plasmids expressing wt or AGF p38α, active MKKs (6E, 3E) or inactive MKKs (6A, 3A), alone or in combination as indicated in the table (**c**). Cell lysates and supernatants were analyzed 30 hrs after transfection by Western blot (**a**, **c**) or ELISA (**b**, **d**). (**e**) Transfected keratinocytes were irradiated with 86.4 mJ/cm^2 UVB and the supernatants were analyzed by ELISA 6 hrs later. Antibodies used for Western blot are indicated. Color code: wild-type p38α-flag (wt), blue; inactive p38α-flag (AGF), yellow; active MKK6E-flag (6E), red; inactive MKK6A-flag (6A), light red; active MKK3E-flag (3E), purple; inactive MKK3A-flag (3A), light purple.

THE P38 DOWNSTREAM TARGET MNK1 CONTRIBUTES TO IL-1β MATURATION IN KERATINOCYTES

Active p38 can translocate to the nucleus to control gene expression via transcription factors activation or trigger cytoplasmic substrates such as MNKs and MKs (Arthur and Ley, 2013). These downstream-activated protein kinases (MAPKAPK) are involved in mRNA translation and stabilization, respectively (Waskiewicz et al., 1997; Kyriakis and Avruch, 2012). A recent study described the role of MNK1 in MSU crystals-induced post-transcriptional production of proIL-1β in human monocytes (Chung et al., 2016b). Unprimed $CD14^{+}$-monocytes do not express proIL-1β; when exposed to MSU, a strong production of proIL-1β was induced and this process was dependent on p38, MNK1 and MK2 as demonstrated by pharmacological inhibition. Interestingly, this effect was not observed in human monocytes-derived macrophages or murine bone marrow-derived macrophages suggesting possible cell-type specificity. Since we demonstrated that active p38 is involved in inflammasome activation in HPKs, we speculated if MNK1 could also be responsible for our observations. Consequently, we exposed keratinocytes to the several concentrations of the MNK1 inhibitors CPG57380 and treat the cells by UVB irradiation or nigericin (Figure 24a). CPG57380 strongly blocked IL-1β secretion with both stimuli and this effect was unaffected also in IFNγ-primed cells (Figure 24b). These preliminary results identify MNK1 as an interesting p38 downstream candidate involved in the maturation of IL-1β upon UVB and nigericin treatment. Further investigations are required to clarify how MNK1 participates to the regulation of inflammasome activation and activity.

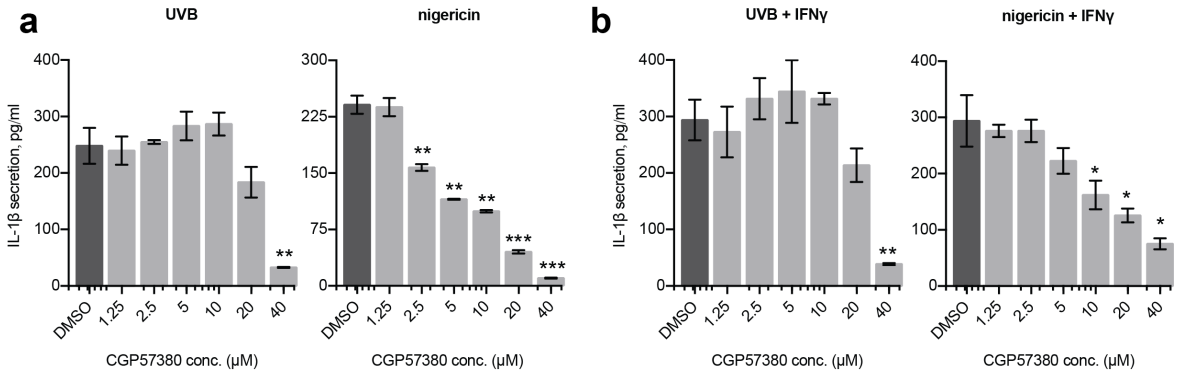

Figure 24 The MNK1 inhibitor CGP57380 blocks IL-1β maturation in HPKs upon UVB and nigericin stimulation

(**a**) Human primary keratinocytes (HPKs) or (**b**) overnight IFNγ-primed (20 ng/ml) HPKs were incubated with the MNK1 inhibitor CGP57380 at the indicated concentrations or with vehicle (DMSO) and exposed to UVB or nigericin 30 min later. Culture supernatants were collected 6 hrs after stimulation and IL-1β secretion was measured by ELISA. Data are expressed as the mean +/- SD and are representative of two independent experiments and analyzed with two-tailed unpaired t-test (**a**-**b**).

3.2 Role of JNK MAPKs in inflammasome activation in myeloid cells

Inhibition of the MAPK JNK in PBMCs and THP-1 impairs the activation of the inflammasome (Figure 10a and c). Our observations were concomitant to the release of a series of studies describing the role of the Syk kinase and JNK in the regulation of the NLRP3 inflammasome (Hara et al., 2013; Okada et al., 2014; Lin et al., 2015). Since we had discrepancies with the published results, we decided to critically revise our experiments. The JNK inhibitor SP600125 does not discriminate between isoforms (Bennett et al., 2001). In order to investigate the role of JNK more precisely, we transduced THP-1 cells with lentiviruses containing shRNA contructs to target JNK1 and JNK2 and the controls lamin A/C and caspase-1. We decided not to investigate the role of JNK3 since it is not expressed in myeloid cells but rather in the brain, heart and testes (Xie et al., 1998). Knockdown efficiency was assessed by Western blot (Figure 25a, b) and by qPCR (Figure 25b) showing a partial downregulation of both JNK isoforms. The expression of inflammasome components was also assessed by qPCR that revealed similar expression levels of NLRP3, ASC, caspase-1 and IL-1β (Figure 25b Figure 25c). After PMA differentiation and ultra-pure LPS (upLPS) priming, we treated the cells with MSU or nigericin to activate the NLRP3 inflammasome. We then analyzed supernatants by Western blot (Figure 25a) and ELISA (Figure 25d). Both caspase-1 and JNK1 shRNA knocked-down THP-1 presented a significant reduction in IL-1β processing with both stimuli when compared to the lamin A/C knockdown cells, whereas JNK2 shRNA knocked-down THP-1 did not affected IL-1β secretion.

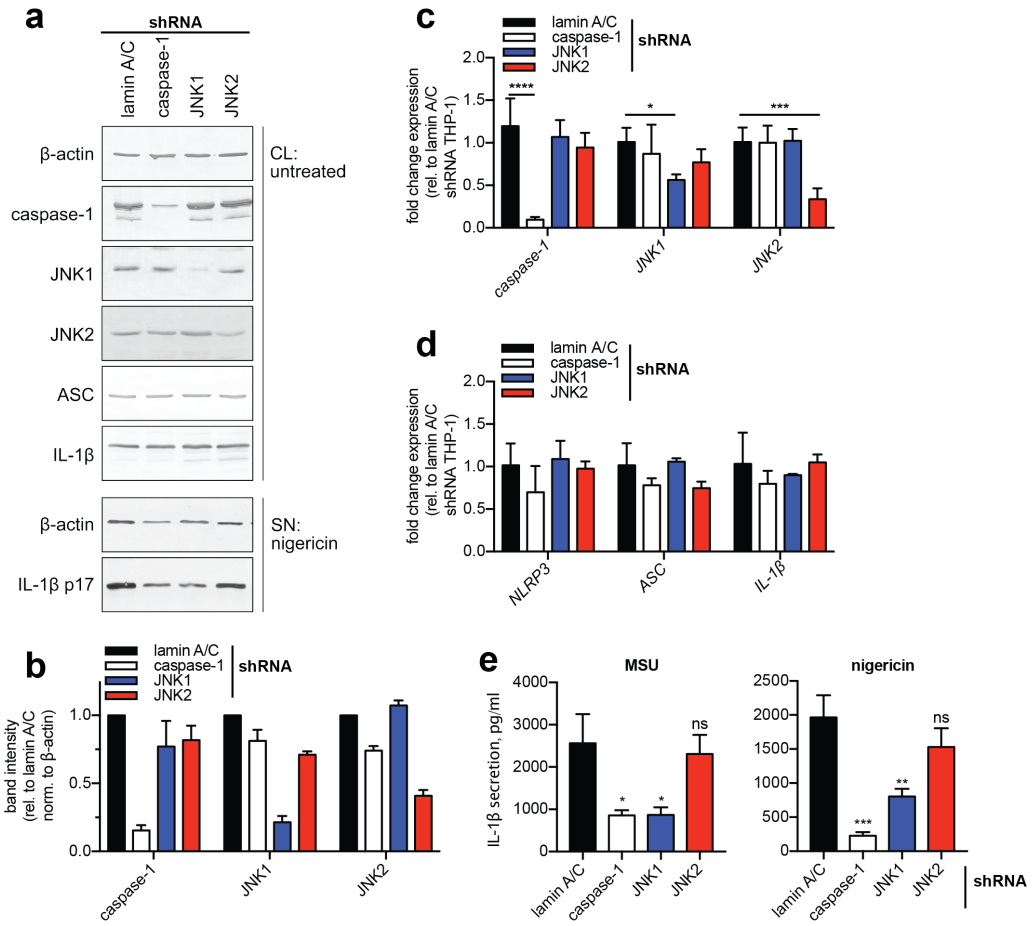

Figure 25 shRNA knockdown of JNK1 resulted in impaired IL-1β secretion in THP-1 cells

(**a**) Western blot analysis and (**b**) bands quantification of cell lysates (CL) and supernatants (SN) from shRNA knocked-down THP-1 differentiated for 3 days with 50 ng/ml PMA, primed overnight with 100 ng/ml upLPS and left untreated (CL) or exposed for 6 hrs to 5 µM nigericin (SN). (**c**, **d**) Fold change in mRNA expression of the indicated genes (x-axis) in shRNA knocked-down THP-1 analyzed by qPCR and relative to the expression in lamin A/C knockdown cells; *RPL27* was used as housekeeper gene. (**e**) IL-1β secretion from PMA-differentiated, upLPS-primed shRNA knocked-down THP-1 exposed to nigericin or MSU (150 µg/ml) for 6 hrs was analyzed by ELISA. shRNA-targeted genes and antibodies used for Western blot are indicated. Data are expressed as the mean +/- SEM of at least two independent experiments with one-way analysis of variance (ANOVA) with Dunnett's multiple-comparison test (**c**, n=2; **e** n=4) or are representative of three independent experiments (**a**).

Next, we wanted to see if activation of JNK promoted inflammasome activation. We therefore generated THP-1 cells that expressed active JNK1 or JNK2 (pJNK1 or pJNK2, respectively) under the control of a doxycycline-inducible promoter by lentiviral transduction. We first assessed the ability of doxycycline to induce pJNKs expression by testing several concentrations for 6 hrs (Figure 26a). At the lowest concentrations, pJNK1 was less expressed than pJNK2 and therefore, we decided to use a concentration of 1 µg/ml for the following experiments. We then treated the cells with doxycycline for different time intervals (Figure 26b) and we identified overnight stimulation (16 hrs) as the best condition since both pJNK1 and pJNK2 were strongly expressed. Moreover, overnight stimulation was able to induce proIL-1β expression in unprimed THP-1 (Figure 26c). Finally, we exposed PMA-differentiated THP-1 to upLPS and/or doxycycline for 16 hrs (Figure 26d). The levels of proIL-1β were not affected by

doxycycline treatment but were increased only in cells primed with upLPS and we could not observe spontaneous IL-1β secretion in the supernatants by ELISA (Figure 26e). We then exposed those unprimed or upLPS-primed THP-1 to nigericin for 6 hrs and assessed the effects of pJNKs by comparing IL-1β secretion. As control, we used THP-1 with inducible expression of eGFP. When unprimed THP-1 exposed to doxycycline overnight were treated with nigericin, pJNK1-overexpressing cells exhibited a significantly higher release of IL-1β compared to eGFP-expressing cells whereas overexpression of pJNK2 had no impact (Figure 26f). In contrast, no significant IL-1β secretion increase could be measured in upLPS-primed cells (Figure 26g). Taken together, these results confirmed JNK1 as a regulator of NLRP3 inflammasome activation in cells of myeloid origin.

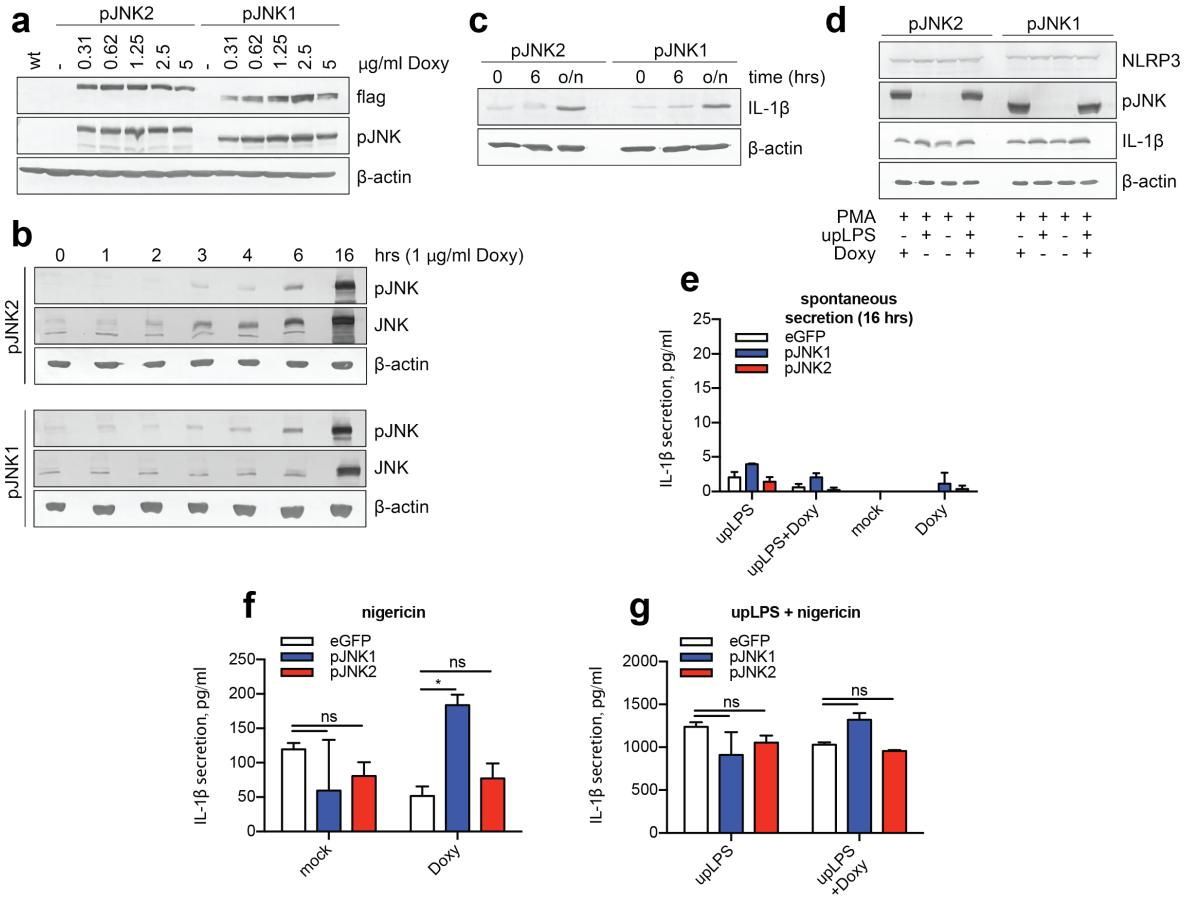

Figure 26 Overexpression of active JNK1 enhances IL-1β release in nigericin-treated THP-1 cells

(**a**-**g**) THP-1 cells were transduced with a lentivirus containing active JNK1 (pJNK1), active JNK2 (pJNK2) or eGFP under the control of a doxycycline-inducible promotor. pJNKs expression was induced in THP-1 cells by different doxycycline doses (**a**) or different time intervals (**b**, **c**) and protein expression was assessed by Western blot. (d-g) pJNKs overexpressing THP-1 were differentiated for 3 days with 50 ng/ml PMA, left untreated or primed overnight with 100 ng/ml upLPS and/or 1 µg/ml doxycycline as indicated; (**d**) protein expression was analyzed by Western blot and spontaneous IL-1β secretion measured 16 hrs after upLpS/doxycyclin stimulation by ELISA; (**f**) unprimed or (**g**) upLPS-primed cells were stimulated for 6 hrs with 5 µM nigericin and supernatants analyzed by ELISA for IL-1β secretion. Antibodies used for Western blot are indicated. Data are expressed as the mean +/- SEM of at two independent experiments with two-way analysis of variance (ANOVA) with Dunnett's multiple-comparison test (**f**, **g**) or are representative of two independent experiments (**a**-**e**).

While the use of gene expression-targeting approaches such as short hairpin RNA allows to study *in vitro* cellular and biochemical processes such as signaling pathways and protein interactions, gene-modified animals consent to investigate these events in the physiological context of a complex organism. JNK knockout mice were originally generated to study T cell differentiation and revealed JNK1 as a negative regulator of T_H2 cytokines and JNK2 involvement in IFNγ production in T_H1 cells (Dong et al., 1998; Yang et al., 1998). We used these JNK knockout mice to investigate the roles of JNK isoforms in inflammasome activation.

At the beginning of this thesis, we obtained conflicting results when performing experiments with JNK1 and JNK2 knockout mice. We suspected some compensatory changes affecting their biological responses and we decided to reimport new animals and repeat the experiments. In order to have proper wild-type control animals and to limit possible compensatory effects, we bred heterozygous animals and used littermates for experiments. We first generated bone marrow-derived macrophages (BMM) from 8 week-old female mice. Caspase-1 knockout animals were used as a control of inflammasome activation deficiency (Guma et al., 2009). BMMs were exposed overnight to upLPS, stimulated with MSU or nigericin for 6 hrs and the supernatants were analyzed for IL-1β secretion by ELISA (Figure 27a). Caspase-1 knockout BMM showed a complete inhibition of IL-1β maturation. JNK1 knockout BMMs also presented a significant reduction of IL-1β maturation with both stimuli in contrast to JNK2 knockout BMMs for which IL-1β secretion was not affected by nigericin treatment.

We then assessed the role of JNK in inflammasome activation *in vivo* with the MSU-induced peritonitis model. Injection of MSU in the peritoneum of mice has, in fact, been associated with an inflammatory response dependent on NLRP3 activation and IL-1β signaling (Chen et al., 2006; Martinon et al., 2006). We injected 1 mg of MSU crystals into the peritoneal cavity of adult mice and, after 3 hrs, we performed a peritoneal lavage and quantified the cells infiltrate by flow cytometry (Figure 27b). The total number of peritoneal exudate cells (PECs) was determined by cell counting with a Neubauer chamber, then PECs were stained for the neutrophil markers Ly6G and 7/4 while infiltrating macrophages were characterized as $CD19^-/CD11c^-/CD11b^+/F4/80^{mid}$ and distinct from the resident peritoneal macrophages ($F4/80^{hi}$) as previously described (Ghosn et al., 2010). JNK1 knockout mice exhibited decreased levels in PECs, neutrophils and macrophages compared to wild-type mice. Even though a trend is also visible for JNK2 knockout mice, these mice presented a significant reduction only in the number of infiltrating neutrophils. These observations confirm our previous *in vitro* results with BMM cells and prove the involvement of JNK1 in a mouse model of inflammasome activation. Altogether, we were able to confirm published observations of the role of JNK, and particularly JNK1, in the regulation of NLRP3 inflammasome activation in myeloid cells of human and murine origin.

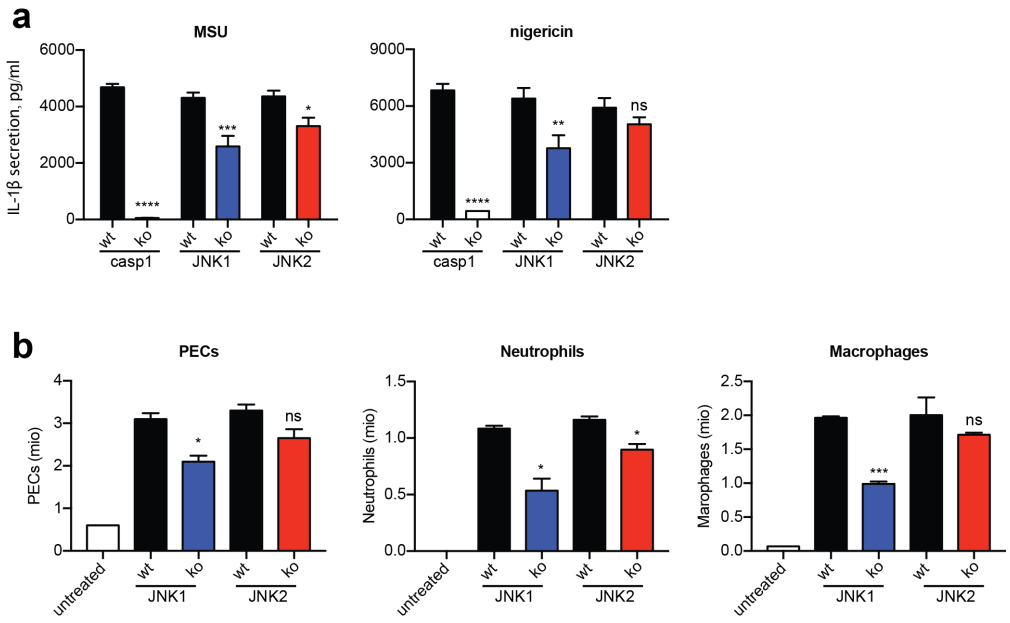

Figure 27 Experiments with BMMs and with the *in vivo* model of MSU-induced peritonitis revealed a role for JNK1 in inflammasome activation in mice

(**a**) Bone-marrow macrophages (BMMs) were generated from *Caspase-1*$^{+/+}$ (casp1 wt), *Caspase-1*$^{-/-}$ (casp1 ko), *JNK1*$^{+/+}$ (JNK1 wt), *JNK1*$^{-/-}$ (JNK1 ko), *JNK2*$^{+/+}$ (JNK2 wt), *JNK2*$^{-/-}$ (JNK2 ko) mice by culturing bone-marrow progenitor cells in 50 ng/ml mM-CSF for 7 days. Cells were then primed overnight with 100 ng/ml upLPS, exposed for 6 hrs to 150 µg/ml MSU or 5 µM nigericin and the supernatants were analysed for IL-1β secretion by ELISA. (**b**) Characterization of infiltrating cells in untreated or JNK1 wt/ko and JNK2 wt/ko mice injected in the peritoneum with 1 mg MSU; 3 hrs after MSU challenge, the peritoneal cavity was washed with PBS and the number of infiltrating peritoneal exudate cells (PECs) was counted with a Neubauer chamber. Cells were further characterized by flow cytometry as follows: neutrophils (Ly6G^{+} / 7/4^{+}), infiltrating macrophages (CD19^{-}/CD11c^{-}/CD11b^{+}/F4/80^{mid}).

3.3 CRISPR/CAS9-MEDIATED GENOME TARGETING IN HUMAN PRIMARY KERATINOCYTES

This project is a collaboration with Serena Grossi, PhD student in the group of PD Dr. Hans-Dietmar Beer. All the experiments were designed, planned and performed together.

In the last 15 years, RNA interference by small interfering RNA (siRNA) and short hairpin RNA (shRNA) have been extensively used to target gene expression. This process plays an important role in antiviral protection in plant and invertebrates (Ding, 2010) but have been described also in mammalian cells (Cullen et al., 2013): viral dsRNA is recognized by the Dicer protein and converted in siRNA to target the viral genome. Similarly, introduction of synthetic siRNA or shRNA into the cells results in the formation of RNA induced silencing complexes (RISCs) which target complementary mRNA for degradation (Carthew and Sontheimer, 2009). Next to zing-finger nucleases (ZFNs), first introduced in 1994 (Klug, 2010), two new biotechnological tools to target gene expression recently became available. These are the Transcription Activator-Like Effectors nucleases (TALENs) and the clustered, regularly interspaced, short palindromic repeats (CRISPR) loci with CRISPR-associated (Cas) genes (CRISPR/Cas) (Gaj et al., 2013). ZFNs and TALENs approaches link nucleases to customizable DNA binding domains (DBDs), hence proteins, that target a specific DNA sequence. Instead, the CRISPR/Cas system only requires a short RNA stretch that targets a specific DNA sequence through RNA-DNA base pairing (Mali et al., 2013). In bacteria and archaea, CRISPR/Cas is part of the adaptive immunity against viral infections and plasmids. In a first phase, these organisms harbour CRISPR loci where they integrate short fragments of viral or plasmid DNA (protospacer) from previous infections (Terns and Terns, 2011). Then, the CRISPR loci, which contains now several protospacer sequences homologous to foreign DNA, are transcribed and processed in short CRISPR RNA (crRNA). crRNA can hybridize to a trans-activating crRNA (tracrRNA) and forms a complex with the nuclease Cas9, which then targets foreign DNA by reinfection (Brouns, 2012). The Cas9 nuclease from *Streptococcus pyogenes* (SpCas9) was engineered into a two-component system composed by the Cas nuclease and the crRNA:tracrRNA fused in a chimeric single-guide RNA (sgRNA) (Jinek et al., 2012). The sgRNA can then be modified by cloning to specifically recognize a 20 nucleotide DNA sequence immediately followed by a 5′-NGG-3′ protospacer-adjacent motif (PAM)[6] (Ran et al., 2013). Targeting of the DNA results in the nuclease-mediated introduction of double-stranded breaks (Figure 28). These are then repaired by homologous recombination repair (HRR), leaving the DNA intact, or by error-prone non-homologous end joining (NHEJ), the latter introducing frame-shift mutations and thus causing

[6] PAM requirements constrain the range of sequences that Cas9 can recognize. This is species specific, therefore Cas9 nucleases from other bacteria have other PAM specificities. Moreover, engineering of SpCas 9 altered its PAM requisite widening the options for more specific genome-editing (Kleinstiver et al., 2015).

premature stop codons which can lead to knockout phenotypes (Valerie and Povirk, 2003). Other than offering a possibility to generate knockout cell lines, ZFNs, TALENs and CRISPR/Cas9 have also a strong relevance in *in vivo* studies since they have been used to generate gene-modified animals (Carbery et al., 2010; Shen et al., 2013; Sung et al., 2013) as alternatives to N-Ethyl-N-Nitrosourea (ENU) and transposon-tagged mutagenesis (Zan et al., 2003; Kitada et al., 2007). As mentioned above, one of the big advantages of the CRISPR/Cas9 technology, is that only a short stretch of guide RNA (sgRNA) is required to specifically target a gene and this system can be easily implemented in the laboratory routine.

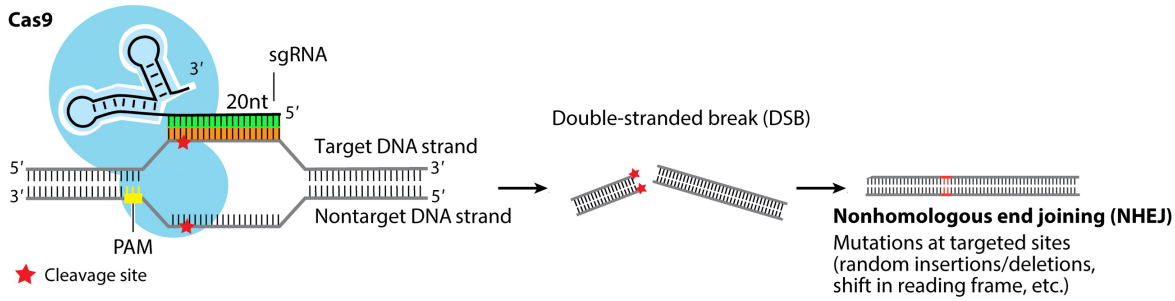

Figure 28 Mechanism of action of engineered CRISPR/Cas9

The Cas9 nuclease forms a complex with the engineered single-guide RNA (sgRNA) to target DNA for cleavage. The sgRNA includes a 20 nucleotides sequence (green) that recognizes the homologous target DNA (orange) immediately followed by a protospacer-adjacent motif (PAM, yellow). Recognition of the target DNA by the CRISPR/Cas9 complex results in the introduction of a double-stranded break which, if repaired by non-homologous end joining, inserts mutations at the targeted site causing premature arrest in the protein translation process. Adapted from (Wang et al., 2016a).

Human primary keratinocytes can be targeted by transfection of siRNA to knockdown genes of interest (Figure 13). However, to target and reduce the expression of very stable proteins, repetitive siRNA transfection and longer culture period are required with consequent stress and toxicity for the cells. We therefore decided to stably target gene expression with the CRISPR/Cas9 technology. Our approach consisted in the transduction of freshly isolated human keratinocytes on feeder cells with lentiviruses carrying a construct that expressed the CRISPR/Cas9 machinery (including the specific sgRNA to target the gene of interest) with a puromycin resistant cassette. Consequently, we also had to generate antibiotic-resistant murine 3T3-J2 feeders, which would allow us to maintain the transduced HPKs in culture for longer period, up to 120-160 divisions (Rasmussen et al., 2013), hence permitting their selection. We isolated HPKs from skin biopsy, preferentially from foreskin of neonatal or infant patients since there is less fat tissues and cells have overall undergone fewer division cycles. Cells were then seeded on a layer of 3T3-J2 feeders previously treated with mitomycin C, a chemotherapeutic agent that impairs proliferation and is used as an alternative to lethal γ-irradiation (Rheinwald, 1980). After isolation, the seeding efficiency of epidermal cells has been reported to be 0.1-1% (Rheinwald and Green, 1975). Therefore, we plated keratinocytes on feeder cells at a 5:1 ratio since most of the

keratinocytes will not adhere. This allowed us to obtain small colonies of proliferating keratinocytes ready to be transduced on day 3 (Figure 29a). After removal of virus-containing medium, the cells were left growing for additional 24-48 hrs without exceeding a confluency of 60-70% to avoid cell differentiation (Poumay and Pittelkow, 1995). Feeder cells were then removed by short incubation with EDTA and keratinocytes were detached by trypsinization and split on new mitomycin-treated 3T3-J2 cells. At this point it is possible to begin the selection of efficiently transduced cells with puromycin. For experiment, CRISPR/Cas9 HPKs (CR-HPKs thereafter) were seeded on mitomycin-c treated 3T3-J2 cells and when they reached 50-60% confluency, feeders were removed with EDTA and CR-HPKs were further cultured in low calcium, keratinocyte-specific serum-free medium (K-SFM) for additional 24 hrs.

In order to establish the best selection conditions, we transduced HPKs with CRISPR/Cas9 constructs expressing sgRNA targeting the protein ASC and, as control, a non-targeting sgRNA. After the first passage we incubated the cells with different concentrations of puromycin (1, 2 and 5 μg/ml) for three weeks and we assessed the knockout efficiency by Western blot (Figure 29b). The highest dose of puromycin tested was well tolerated and resulted in 80-90% decrease of ASC protein levels in targeted cells after 1 week (Figure 29c). Longer selection at this antibiotic dose, did not further affect ASC protein levels while the lower doses of puromycin improved the ASC knockout efficiency with additional selection time. We therefore established a successful transduction and selection strategy to target gene expression in human primary keratinocytes using the CRISPR/Cas9 technology.

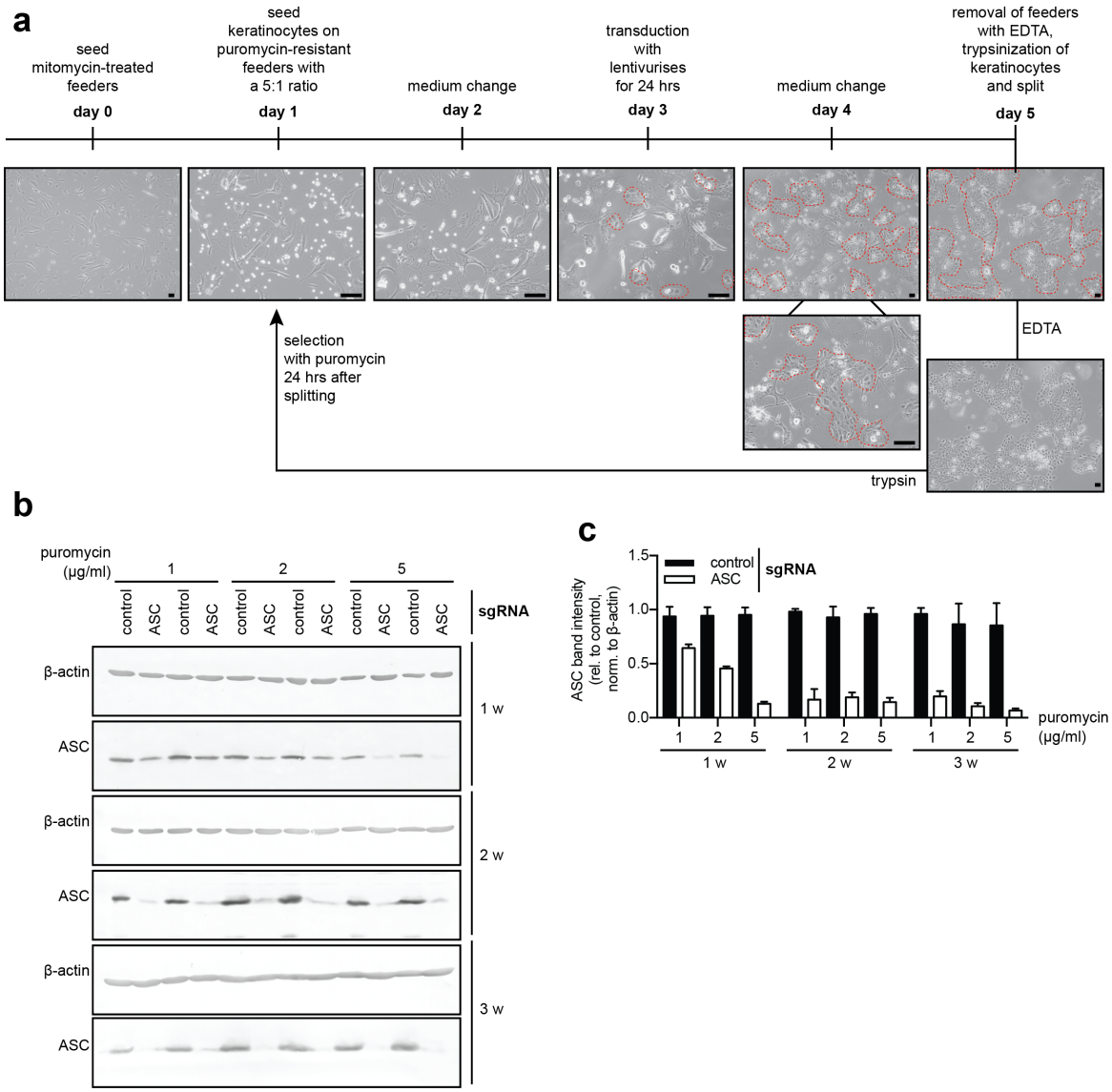

Figure 29 Generation and selection of CRISPR/Cas9-targeted human primary keratinocytes

(**a**) Schematic protocol for the generation of CRISPR/Cas9-targeted HPKs. Cells are co-cultured on mitomycin-treated 3T3-J2 feeder cells and infected with a lentivirus expressing Cas9 and sgRNA including the DNA target sequence. Scale bars, 100 µM; red dotted lines contour keratinocytes clones. (**b**, **c**) Western blot analysis and quantification of ASC bands from lysate of HPKs transduced with CRISPR/Cas9 targeting ASC or a non-targeting sgRNA (control) and cultured under puromycin selection at the indicated doses and periods (in weeks). (**c**) ASC bands intensity are normalized on β-actin protein levels and relative to control sgRNA CR-HPKs. sgRNA targeted genes and antibodies used for Western blot are indicated.

CRISPR/CAS9-TARGETED KERATINOCYTES POSSESS A FUNCTIONAL INFLAMMASOME

Next, we wanted to apply the CRISPR/Cas9 technology to our studies of the regulation of inflammasome activation in keratinocytes. To this end, we generated ASC and caspase-1 knockout HPKs as described above. In addition to the non-targeting sgRNA CR-HPKs control, we maintained wild-type non-transduced cells on 3T3-J2 feeder cells as long as CRISPR-targeted cells.

Cells were then seeded for experiments and left untreated or irradiated with UVB. Western blot revealed a reduced expression of sgRNA-targeted proteins while proIL-1β expression remained unaffected (Figure 30a). Supernatants of UVB-irradiated cells were then analyzed by Western blot (Figure 30a) and ELISA (Figure 30b) and compared to wild-type non-transduced cells. Caspase-1 and ASC sgRNA-targeted cells showed decreased release of mature IL-1β whereas the secretion of control non-targeting sgRNA CR-HPKs was comparable to wild-type cells maintained on feeders. These results, together with the experiments where p38 was deleted (Figure 16), show that CRISPR\Cas9 technology can successfully be used for studies of inflammasome activation in HPKs.

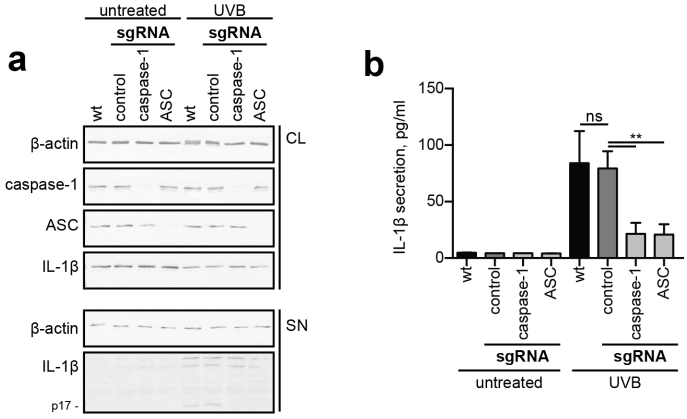

Figure 30 ASC and caspase-1 sgRNA CRISPR/Cas9-targetd HPKs have impaired inflammasome activation

(**a**) Western blot analysis from cell lysate of non-targeting control, caspase-1 and ASC sgRNA CRISPR/Cas9-targetd HPKs or wild-type cells maintained on feeder cells. (**b**) ELISA for IL-1β secretion in supernatants of untreated and UVB irradiated control, caspase-1 and ASC sgRNA CR-HFKs and HPKs. sgRNA targeted genes and antibodies used for Western blot are indicated. Data are expressed as the mean +/- SEM of three independent experiments with two-way analysis of variance (ANOVA) with Dunnett's multiple-comparison test (**b**) or are representative of three independent experiments (**a**).

CRISPR/CAS9-TARGETED KERATINOCYTES ARE ABLE TO DIFFERENTIATE *IN VITRO*

Our approach to directly target primary keratinocytes using CRISPR/Cas9 technology potentially opens opportunities for skin engineering and gene therapy. A recent publication reported the successful engraftment of artificial skin made from patient-derived keratinocytes transduced by a retroviral vector expressing a laminin subunit (*LAMB3*) to treat junctional epidermolysis bullosa (Bauer et al., 2017). Other groups applied the CRISPR/Cas9 technology to human fibroblast which were then re-differentiated into keratinocytes from induced pluripotent stem cells (iPSC) (Shinkuma et al., 2016; Webber et al., 2016). A prerequisite for the generation of skin substitutes is the ability of the engineered keratinocytes to differentiate. In order to test the differentiation potential of our CR-HPKs, we cultured control and ASC sgRNA-targeted cells in K-SFM medium until they reached confluency and afterwards for a week in Ca^{2+}-containing differentiation medium (DM). We then assessed by Western blot the expression of several differentiation markers such as suprabasal keratin 1 and 10, granular layer filaggrin and the terminal differentiation marker involucrin (Figure 31). Both control and ASC sgRNA-targeted CR-HFKs expressed increasing levels of keratin 1 from the first day in DM whereas keratin 10 was strongly induced from day 3. The late stage markers filaggrin and involucrin were expressed from day 5 while keratin 14 was always detected suggesting the persistence of undifferentiated cells.

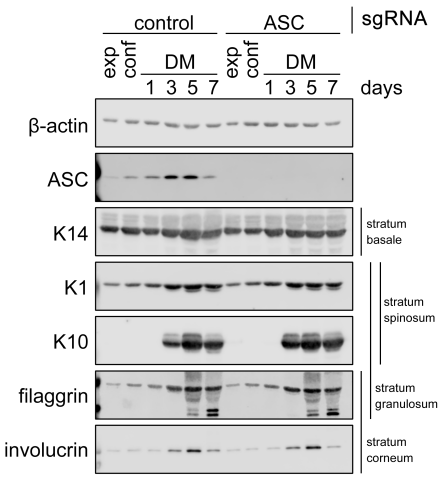

Figure 31 CRISPR/Cas9-targeted keratinocytes are able to differentiate *in vitro*

Western blot analysis from cell lysate of control and ASC sgRNA CRISPR/Cas9-targetd HPKs in the exponential growth phase (exp) and at cell confluency (conf) in normal keratinocytes medium (K-SFM) or at the indicated days after having switched to keratinocyte differentiation medium (DM). Antibodies used for Western blot are indicated on the left of the blots. The *in vivo*, physiological expression locations of the differentiation markers are indicated on the right.

Overall, we established a protocol to significantly target gene expression in human primary keratinocytes with the CRISPR/Cas9 technology; these cells possess a functional inflammasome and maintain full *in vitro* differentiation potential. This method opens therefore new perspectives for the generation of gene-modified skin equivalents for the treatment of disabling conditions such as epidermolysis bullosa or burn wounds.

CHAPTER 4: DISCUSSION AND CONCLUSIONS

The inflammasomes are important intracytoplasmic innate immune mediators that induce inflammation in response to pathogen- and danger-associated molecular patterns (Latz et al., 2013). While inflammasome-mediated activation has been heavily studied in myeloid immune cells including macrophages and dendritic cells, the molecular mechanisms involved in inflammasome activation and IL-1β secretion by keratinocytes have been less investigated. In this thesis, we provide evidence that, in human keratinocytes, inflammasome activity and subsequent IL-1β secretion critically depend on the p38 MAPK, notably the p38α and p38δ isoforms

Several kinases have been involved in the regulation of inflammasome activity by direct interaction or by phosphorylation of NLRs (Qu et al., 2012; He et al., 2016). Syk and the MAPK JNK were shown to target the adaptor protein ASC for phosphorylation and control its oligomerization in myeloid cells (Hara et al., 2013; Okada et al., 2014; Lin et al., 2015). We could show that the pharmacological inhibition of the JNK and Syk kinases also blocks the activation of the inflammasome in human primary keratinocytes (HPKs) but, most importantly, we observed a strong inhibition of IL-1β maturation when we targeted the p38 MAPK. This effect could not be observed in myeloid cells suggesting the existence of a certain cell type specificity regarding the nature of the MAPK involved in inflammasome activation. In fact, exposure of human PBMCs and THP-1 cells to p38 inhibitors before MSU or nigericin exposure did not impact IL-1β secretion. Instead, when we treated keratinocytes with JNK and Syk inhibitors, we also blocked the maturation of this cytokine. Myeloid cells are able to secrete high levels of IL-1β within minutes after exposure to inflammasome activators such as ATP (Mariathasan et al., 2006). In contrast, release of mature IL-1β in the supernatant of UVB-irradiated HPKs can be detected only after 3 hours (Sollberger et al., 2012). When we exposed keratinocytes to p38 inhibitors one hour after UVB irradiation, we still observed a strong impairment in IL-1β secretion. The Syk inhibitor piceatannol had even a stronger impact since a late treatment (up to 4 hours after stimulation) of HPKs with this inhibitor resulted in a partial IL-1β inhibition. Surprisingly, we observed that JNK inhibitor given before UVB irradiation could block IL-1β secretion by keratinocytes, whereas it had no impact on IL-1β secretion when given at the time of irradiation or at later time points. These observations suggest that Syk and, to some extent, JNK are also involved in the regulation of inflammasome activation in keratinocytes. However, the fact that inhibiting JNK at the time of UVB irradiation or later is inefficient suggests that p38, but also Syk, are the main drivers of inflammasome activation in keratinocytes once the cells are exposed to danger signals. The use of pharmacological inhibitors allowed us to identify different kinases involved in inflammasome activation. However, the high sequence homology between kinase isoforms of the same family but also the similarity of the conserved active site among MAPKs impede the development of selective inhibitors. Among the tested p38 inhibitors, in fact, only VX-702 is able to target a single isoform (p38α) whereas p38 inhibitor I and SB203580 block p38α and β and BIRB 796 all four p38 isoforms but also JNK2 (Bain et al., 2007). Therefore, it was important to target the different isoforms through RNA interference or gene deletion.

When compared to myeloid immune cells, the specific characteristic of human primary keratinocytes with respect to inflammasome activation resides in the specific requirement of p38 activation for the maturation of IL-1β. Moreover, myeloid cells require a priming stimulus to induce the expression of inflammasome components and particularly NLRP3 and proIL-1β (Bauernfeind et al., 2009), whereas HPKs express constitutively proIL-1α, proIL-1β and proIL-18 (Feldmeyer et al., 2007). Interestingly, it has recently been shown that, in $CD14^+$ monocytes, p38 regulates MSU-induced IL-1β maturation and secretion at the post-transcriptional level (Chung et al., 2016b). Our observation that neither pharmacological p38-inhibitors nor p38 gene silencing resulted in a reduction of pro-IL-1β levels in keratinocytes is not compatible with such a regulatory mechanism in keratinocytes. On the other hand, inhibition of the p38 target MNK1, known to be involved in mRNA translation (Waskiewicz et al., 1997), also resulted in the abrogation of IL-1β secretion suggesting that this MAPK-activated protein kinase is also involved in inflammasome activation in keratinocytes but its precise role remains to be clarified. Investigation of *de novo* protein synthesis in HPKs is difficult since the treatment with cycloheximide, an eukaryotic protein synthesis inhibitor, results in IL-1β maturation (Feldmeyer et al., 2007). The opposite approach consists in the overexpression of components involved in inflammasome activation by stimulation of HPKs with IFNγ. In these conditions, HPKs showed increased maturation of IL-1β upon UVB irradiation; nevertheless, the treatment with p38 inhibitors, but also MNK1, caused a substantial decrease in the levels of IL-1β secretion supporting our conclusion that p38 and MNK1 regulate inflammasome activity in keratinocytes also in a mechanism independent of the regulation of RNA translation.

At the steady state, the levels of NLRP1 expression in HPKs and primary fibroblasts are similar to those measured in PBMCs (Zhong et al., 2016). We found that keratinocytes NLRP3 mRNA levels were 100-fold reduced when compared to NLRP1 and we could detect NLRP3 protein expression only after IFNγ stimulation. Interestingly, when we silenced NLRP1 in HPKs with siRNA or by CRISPR/Cas9 in the keratinocyte cell line KerTr, we observed a strong inhibition in IL-1β secretion upon UVB irradiation whereas, when we targeted NLRP3 we could not observe such an inhibition. Surprisingly, we also obtained a reduction when NLRP1-targeted cells were stimulated with the pore-forming toxin nigericin, that is thought to be a selective NLPR3 activator (Mariathasan et al., 2006). When we exposed the cells to the NLRP3 specific inhibitor MCC950 we could only observe a marginal decrease in IL-1β secretion upon both UVB and nigericin exposure. MCC950 has been shown to block specifically NLRP3 activation without affecting NLRP1, AIM2 and NLRC4 inflammasomes (Coll et al., 2015). These observations indicate that NLRP1, rather than NLRP3, plays a major role in the response to UVB irradiation but also to nigericin. The importance of NLRP1 activity in keratinocytes is also supported by a recent study that described a germline mutation in the inflammasome causing extensive inflammation in the skin and susceptibility to the development of skin cancer (Zhong et al., 2016).

Currently, no specific agonist of human NLRP1 has been identified. MDP was originally described to bind and activate NLRP1 in a cell-free system (Faustin et al., 2007) but its main function consists in the

activation of the cytosolic sensor NOD2 (Girardin et al., 2003; Hsu et al., 2008) but also NLRP3 (Martinon et al., 2004; Kovarova et al., 2012). In contrast, murine NLRP1 can be activated by the *Bacillus anthracis* lethal toxin (LT) via proteolysis of the N-terminal portion of the protein that is absent in human (Chavarria-Smith et al., 2016). LT was reported to increase NLRP1-dependent caspase-1 activity in the human THP-1 cell line but no evidence of IL-1β release has been reported (Zhai et al., 2017). Moreover, LT acts by inhibiting the functions of MAP2Ks, hence blocking ERKs, JNKs and p38 MAPKs and so paralyzing the host's innate immune response (Duesbery et al., 1998; Park et al., 2002). This makes LT an inappropriate alternative to UVB and nigericin as NLRP1 activator for our research. CRISPR/Cas9 sgRNA targeting NLRP1 and NLRP3 in keratinocytes would help support our observations that identify UVB as a first specific activator of human NLRP1 and, at the same time, elucidate which NLR(s) is/are triggered by nigericin in keratinocytes.

There are an increasing number of studies providing evidence that p38 plays a key role in the pathogenesis of a growing number of chronic inflammatory diseases including inflammatory skin diseases and, consequently, that p38, as key signaling molecule, may present as a promising therapeutic target in such diseases (Kumar et al., 2003; Cuenda and Rousseau, 2007). For instance, it has been reported that the kinase activity of p38 MAPKs, including the isoforms α, β and δ, are increased in the lesional epidermis of patients suffering from psoriasis (Johansen et al., 2005; Yu et al., 2007), and that inhibition of p38 suppresses the development of psoriasis-like lesions in a human skin transplant model of psoriasis (Mihara et al., 2012). p38 MAPKs also play an important role in inflammation induced by UVB where they have been shown to exhibit increased activity in human and mouse keratinocytes (Chen and Bowden, 1999; Chen et al., 1999), and where UVB has been shown to induce IL-6 and IL-8 expression in keratinocytes in a cyclooxygenase-2 (COX-2)- and p38-dependent manner (Chen et al., 2001; Kim et al., 2005). Moreover, COX-2 can regulate inflammasome activity by enhancing NLRP3 and IL-1β expression upon LPS stimulation and by increasing the damaging of mitochondria which results in augmented ROS production and release of mtDNA in the cytosol (Hua et al., 2015). The role of p38 in inflammation has also been extensively studied in mouse models. UVB irradiation of hairless mice causes strong inflammatory responses with development of p38-dependent erythema (Kim et al., 2005). Mice with an epithelial tissue-specific p38α knockout ($Mapk14^{fl/fl}$ x K14-Cre) showed less neutrophil infiltration upon UVB irradiation (Kim et al., 2008). However, evidences from our department indicate that murine keratinocyte do not express proIL-1β and do not have a functional inflammasome (Sand et al., submitted manuscript), suggesting that the involvement of p38 in inflammation in murine skin is limited to its activity in gene expression regulation via COX-2 rather than our described inflammasome regulatory mechanism.

Murine and human skin are indeed different. Mice are wild nocturnal animals, they are not exposed to UV and their skin is completely cover by fur. The epidermis and dermis are thinner, packed with hair follicles and, contrarily to human, dendritic epidermal T cells are found in the epidermis (Pasparakis et al., 2014). Activation of the inflammasome in mouse keratinocytes is controversial. Some studies have

suggested that the main source of IL-1β in the mouse skin are myeloid leukocytes and Langerhans cells (Schreiber et al., 1992; Shornick et al., 2001; Matsushima et al., 2010). Others, instead, have reported that murine primary keratinocytes can secrete IL-1β when treated with the contact sensitizer trinitrochlorobenzene or irradiated with UVB (Watanabe et al., 2007; MacLeod et al., 2014). Controversially, it was shown that NLRP3 and IL-1β secretion are not detected in primary murine keratinocyte (Guarda et al., 2011). The TLR7 agonist imiquimod was described to activate the NLRP3 inflammasome (Gross et al., 2016) and its application on murine skin induced an inflammatory response dependent on IL-1 and Myd88 but independent of the inflammasome (Rabeony et al., 2015). Mice are therefore inadequate to study inflammasome activation in keratinocytes *in vivo* but remain a model of interest for the study of the role of myeloid cells in skin inflammation. In fact, next to the inhibition of IL-1β secretion in THP-1 cells with JNK inhibitors and with shRNA to JNK1, we could use knockout animals to generate bone marrow-derived macrophages (BMMs). Treatment of JNK1-deficient BMMs with NLRP3 activators resulted in impaired inflammasome activity and mice lacking JNK1 were protected in the MSU-induced peritonitis model, showing decreased infiltration of neutrophils and inflammatory macrophages in the peritoneal cavity. These observations confirmed recent published studies describing the role of JNK and Syk, in inflammasome regulation (Hara et al., 2013; Okada et al., 2014). It is also important to notice that we could observe a minor impact on inflammasome activity in JNK2 knockout mice. Similarly, when we knocked down p38 in HPKs we clearly identified p38α and δ as the main isoforms involved. This suggests that the redundancy of functions among the different isoforms could compensate the deletion of one member (Saba-El-Leil et al., 2016).

Our results with JNK and p38 inhibition, gene silencing or deletion, were supported by the increased levels of mature IL-1β measured in cells overexpressing active MAPKs. In keratinocytes, we observed spontaneous secretion of IL-1β when p38α was overexpressed with active MKK6. Interestingly, overexpression of active MKK6 alone or p38α with MKK3 did not result in the same effect. In THP-1 cells, the induction of active JNK1 resulted in increased maturation of IL-1β only when unprimed cells were challenged with nigericin whereas, when the cells were also exposed to LPS, the increase was not significant suggesting that JNK1 participates also in the priming phase. With our recent progresses in the culture, transduction and selection of HPKs, a lentiviral approach to overexpress proteins in an inducible manner can be applied to this cell type. This would allow to overcome possible activation of the AIM2 inflammasome by transfection of plasmids (Dombrowski et al., 2011) but also to perform rescue experiments in p38-sgRNA targeted cells. Active p38 expression could be induced independently of UVB irradiation or nigericin treatment, thus allowing to understand the temporal involvement of the kinase in the activation of the inflammasome.

Overexpression of active p38 in HPKs was also employed to study phosphorylation of ASC in keratinocytes but none of the tested approaches permitted to detect it. ASC phosphorylation was first described in murine macrophages and linked to the Syk and JNK activities (Hara et al., 2013). Recently, it was shown that Syk activates Pyk2, another tyrosine kinase able to directly interact and phosphorylate

overexpressed human ASC (Chung et al., 2016a). To our knowledge, there are no studies documenting the phosphorylation of endogenous ASC in human cells. In our approach, we first specifically tried to identify phosphorylation of ASC on its tyrosine residue then, with PhosTag based mobility-shift assay, we included all possible phospho-residues. With both methods, we could not detect this post-translation modification. Immunoprecipitation may not be sensible enough to detect a small fraction of phosphorylated protein. Nevertheless, we also analyzed by mobility-shift assay samples containing enriched oligomerized ASC and, also in this case, phosphorylation could not be observed. Proteomics is an essential part of modern life science research and has been used in several occasions to study the inflammasome (Nyman et al., 2017). In fact, the NLRP3 interaction partner NEK7 and the pyroptotic protein gasdermin D were identified by affinity purification followed by mass-spectrometry analysis (He et al., 2015; He et al., 2016). A similar approach linked to phosphoproteomics could be therefore used to further investigate whether in humans, and especially in HPKs, ASC is phosphorylated during inflammasome activation.

Even if could not show its phosphorylation upon irradiation of HPKs with UVB, ASC oligomerizes in a p38-dependent manner. ASC oligomerization can be used as tool to identify inflammasome activation (Stutz et al., 2013; Sester et al., 2015; Beilharz et al., 2016). When we target p38 by pharmacological inhibition, siRNA and CRISPR/Cas9, we blocked the formation of oligomers as measured by Western blot after chemical cross-linking. Oligomerization of ASC can also be detected by immunofluorescence. Several groups have generated cell lines or transgenic mice expressing fluorescent labeled ASC (Tzeng et al., 2016) and could show the formation of ASC specks after stimulation of cells with inflammasome activators. In our approach, we detected specks formed by endogenous ASC, thus limiting the possibilities of auto-oligomerization that the overexpression of ASC can cause (Hoss et al., 2017). Therefore, this could be used as a valid readout for inflammasome activation in keratinocytes. Again, p38 inhibition, gene silencing or deletion, resulted in decreased numbers of ASC specks. The latter were always localized in close proximity to the nucleus. Interestingly, also NLRP1 was observed in proximity to the nucleus of PMA-treated THP-1 supporting the role of this NLR in UVB-irradiated keratinocytes (Zhai et al., 2017).

Thanks to work of Prof. Charpentier and Prof. Zhang, the CRISPR/Cas9 technology is now accessible to several laboratories (Jinek et al., 2012; Ran et al., 2013). We established a method that enabled us to directly target gene expression in HPKs and subsequently select and expand them. We could show that HPKs transduced with non-targeting sgRNA were able to activate the inflammasome and secrete mature IL-β in a comparable manner to wild-type HPKs. When we targeted the two components of the inflammasome ASC and caspase-1 but also p38α, we instead observed a reduction in IL-β secretion. As additional control, we would need to delete a protein that is not involved in inflammasome activity. Moreover, the deletion of NLR genes in HPKs will help us answering the open questions about the role of NLRP1 in UVB irradiation and particularly in the response to nigericin.

In addition to their use to study the inflammasome, our CRISPR/Cas9 targeted HPKs were able to differentiate *in vitro*, thus opening application possibilities such as the generation of skin substitutes (Biedermann et al., 2013). Skin diseases such as epidermolysis bullosa simplex and epidermolytic hyperkeratosis are linked to mutations in either of the *keratin 5* or *keratin 14* genes and *keratin 1* or *keratin 10*, respectively (Oji et al., 2016). Clinical features include skin blistering due to the fragility of the basal layer cells or skin thickening due to cellular hyperproliferation and accumulation of corneocytes (Rothnagel et al., 1992; McLean and Moore, 2011). No cure is available and treatments are symptomatic. Recently, CRISPR/Cas9 technology was used to correct mutations causing recessive systrophic epidermolysis bullosa: primary fibroblasts were isolated from a patient, modified by CRISPR/Cas9, used to derive induced pluripotent stem cells (iPSCs) and subsequently differentiated into keratinocytes (Webber et al., 2016). Our approach, instead, by targeting directly primary keratinocytes requires less manipulations of the cells and is faster. In this work, we infected keratinocytes with lentiviruses expressing the CRISPR/Cas9 construct with the sgRNA of interest. Of note, lentiviruses integration in the human genome can disrupt genetic information resulting in aberrant transcripts (Moiani et al., 2012). Moreover, the CRISPR/Cas9 machinery is constantly expressed and this could lead to off-target effect with time. It is therefore of fundamental importance to select sgRNA sequences with low off-target prediction score (Marx, 2014). Other approaches, such as cell transfection with plasmids expressing the CRISPR/Cas9 machinery or transfection of the recombinant Cas9 protein with the sgRNA of interest, are currently available but have the disadvantage of lacking selection possibilities on the long term since their expression is transient. As an effort to eliminate xenogeneic cells from our method, murine 3T3-J2 feeder cells should be substituted with human fibroblast (Jubin et al., 2011). Improvement of this protocol may generate targeted cells with the ability to create skin substitutes that can be used as skin replacement therapy (Marino et al., 2014).

In conclusion, this work identified p38 MAPKs as key players in inflammasome activity in HPKs and NLRP1 as a key sensor of external stressors such as UV light and bacterial nigericin in the epidermis. Therefore, in addition to IL-1β, both p38 and NLRP1 constitute potential targets for the treatment of inflammatory skin diseases involving keratinocytes.

REFERENCES

Adam, C., Wohlfarth, J., Haussmann, M., Sennefelder, H., Rodin, A., Maler, M., et al. (2017). Allergy-Inducing Chromium Compounds Trigger Potent Innate Immune Stimulation Via ROS-Dependent Inflammasome Activation. *J Invest Dermatol* 137(2)**,** 367-376. doi: 10.1016/j.jid.2016.10.003.

Adler, V., Schaffer, A., Kim, J., Dolan, L., and Ronai, Z. (1995). UV irradiation and heat shock mediate JNK activation via alternate pathways. *J Biol Chem* 270(44)**,** 26071-26077.

Afonina, I.S., Muller, C., Martin, S.J., and Beyaert, R. (2015). Proteolytic Processing of Interleukin-1 Family Cytokines: Variations on a Common Theme. *Immunity* 42(6)**,** 991-1004. doi: 10.1016/j.immuni.2015.06.003.

Afonina, I.S., Tynan, G.A., Logue, S.E., Cullen, S.P., Bots, M., Luthi, A.U., et al. (2011). Granzyme B-dependent proteolysis acts as a switch to enhance the proinflammatory activity of IL-1alpha. *Mol Cell* 44(2)**,** 265-278. doi: 10.1016/j.molcel.2011.07.037.

Afshar, M., and Gallo, R.L. (2013). Innate immune defense system of the skin. *Vet Dermatol* 24(1)**,** 32-38 e38-39. doi: 10.1111/j.1365-3164.2012.01082.x.

Aglietti, R.A., and Dueber, E.C. (2017). Recent Insights into the Molecular Mechanisms Underlying Pyroptosis and Gasdermin Family Functions. *Trends Immunol* 38(4)**,** 261-271. doi: 10.1016/j.it.2017.01.003.

Akdis, M., Aab, A., Altunbulakli, C., Azkur, K., Costa, R.A., Crameri, R., et al. (2016). Interleukins (from IL-1 to IL-38), interferons, transforming growth factor beta, and TNF-alpha: Receptors, functions, and roles in diseases. *J Allergy Clin Immunol* 138(4)**,** 984-1010. doi: 10.1016/j.jaci.2016.06.033.

Aksentijevich, I., Masters, S.L., Ferguson, P.J., Dancey, P., Frenkel, J., van Royen-Kerkhoff, A., et al. (2009). An autoinflammatory disease with deficiency of the interleukin-1-receptor antagonist. *N Engl J Med* 360(23)**,** 2426-2437. doi: 10.1056/NEJMoa0807865.

Aksentijevich, I., Nowak, M., Mallah, M., Chae, J.J., Watford, W.T., Hofmann, S.R., et al. (2002). De novo CIAS1 mutations, cytokine activation, and evidence for genetic heterogeneity in patients with neonatal-onset multisystem inflammatory disease (NOMID): a new member of the expanding family of pyrin-associated autoinflammatory diseases. *Arthritis Rheum* 46(12)**,** 3340-3348. doi: 10.1002/art.10688.

Allen, I.C., Wilson, J.E., Schneider, M., Lich, J.D., Roberts, R.A., Arthur, J.C., et al. (2012). NLRP12 suppresses colon inflammation and tumorigenesis through the negative regulation of noncanonical NF-kappaB signaling. *Immunity* 36(5)**,** 742-754. doi: 10.1016/j.immuni.2012.03.012.

Altiok, E., Aksoy, F., Perk, Y., Taylan, F., Kim, P.W., Ilikkan, B., et al. (2012). A novel mutation in the interleukin-1 receptor antagonist associated with intrauterine disease onset. *Clin Immunol* 145(1)**,** 77-81. doi: 10.1016/j.clim.2012.08.003.

Arthur, J.S., and Ley, S.C. (2013). Mitogen-activated protein kinases in innate immunity. *Nat Rev Immunol* 13(9)**,** 679-692. doi: 10.1038/nri3495.

Ascierto, P.A., Kirkwood, J.M., Grob, J.J., Simeone, E., Grimaldi, A.M., Maio, M., et al. (2012). The role of BRAF V600 mutation in melanoma. *J Transl Med* 10**,** 85. doi: 10.1186/1479-5876-10-85.

Ashwell, J.D. (2006). The many paths to p38 mitogen-activated protein kinase activation in the immune system. *Nat Rev Immunol* 6(7)**,** 532-540. doi: 10.1038/nri1865.

Atianand, M.K., and Harton, J.A. (2011). Uncoupling of Pyrin-only protein 2 (POP2)-mediated dual regulation of NF-kappaB and the inflammasome. *J Biol Chem* 286(47)**,** 40536-40547. doi: 10.1074/jbc.M111.274290.

Bachetti, T., and Ceccherini, I. (2014). Tumor necrosis factor receptor-associated periodic syndrome as a model linking autophagy and inflammation in protein aggregation diseases. *J Mol Med (Berl)* 92(6)**,** 583-594. doi: 10.1007/s00109-014-1150-5.

Bain, J., Plater, L., Elliott, M., Shpiro, N., Hastie, C.J., McLauchlan, H., et al. (2007). The selectivity of protein kinase inhibitors: a further update. *Biochem J* 408(3)**,** 297-315. doi: 10.1042/BJ20070797.

Ball, N.J., Yohn, J.J., Morelli, J.G., Norris, D.A., Golitz, L.E., and Hoeffler, J.P. (1994). Ras mutations in human melanoma: a marker of malignant progression. *J Invest Dermatol* 102(3)**,** 285-290.

Baroja-Mazo, A., Martin-Sanchez, F., Gomez, A.I., Martinez, C.M., Amores-Iniesta, J., Compan, V., et al. (2014). The NLRP3 inflammasome is released as a particulate danger signal that amplifies the inflammatory response. *Nat Immunol* 15(8)**,** 738-748. doi: 10.1038/ni.2919.

Bauer, J.W., Koller, J., Murauer, E.M., De Rosa, L., Enzo, E., Carulli, S., et al. (2017). Closure of a Large Chronic Wound through Transplantation of Gene-Corrected Epidermal Stem Cells. *J Invest Dermatol* 137(3)**,** 778-781. doi: 10.1016/j.jid.2016.10.038.

Bauernfeind, F.G., Horvath, G., Stutz, A., Alnemri, E.S., MacDonald, K., Speert, D., et al. (2009). Cutting edge: NF-kappaB activating pattern recognition and cytokine receptors license NLRP3 inflammasome activation by regulating NLRP3 expression. *J Immunol* 183(2)**,** 787-791. doi: 10.4049/jimmunol.0901363.

Beer, H.D., Contassot, E., and French, L.E. (2014). The inflammasomes in autoinflammatory diseases with skin involvement. *J Invest Dermatol* 134(7)**,** 1805-1810. doi: 10.1038/jid.2014.76.

Beer, H.D., Munding, C., Dubois, N., Mamie, C., Hohl, D., and Werner, S. (2002). The estrogen-responsive B box protein: a novel regulator of keratinocyte differentiation. *J Biol Chem* 277(23)**,** 20740-20749. doi: 10.1074/jbc.M111233200.

Beeson, P.B. (1948). Temperature-elevating effect of a substance obtained from polymorphonuclear leucocytes. *J Clin Invest* 27(4)**,** 524.

Beilharz, M., De Nardo's, D., Latz, E., and Franklin, B.S. (2016). Measuring NLR Oligomerization II: Detection of ASC Speck Formation by Confocal Microscopy and Immunofluorescence. *Methods Mol Biol* 1417**,** 145-158. doi: 10.1007/978-1-4939-3566-6_9.

Belkaid, Y., and Hand, T.W. (2014). Role of the microbiota in immunity and inflammation. *Cell* 157(1)**,** 121-141. doi: 10.1016/j.cell.2014.03.011.

Bennett, B.L., Sasaki, D.T., Murray, B.W., O'Leary, E.C., Sakata, S.T., Xu, W., et al. (2001). SP600125, an anthrapyrazolone inhibitor of Jun N-terminal kinase. *Proc Natl Acad Sci U S A* 98(24)**,** 13681-13686. doi: 10.1073/pnas.251194298.

Berger, R., Smit, G.P., Schierbeek, H., Bijsterveld, K., and le Coultre, R. (1985). Mevalonic aciduria: an inborn error of cholesterol biosynthesis? *Clin Chim Acta* 152(1-2)**,** 219-222.

Bergsbaken, T., Fink, S.L., and Cookson, B.T. (2009). Pyroptosis: host cell death and inflammation. *Nat Rev Microbiol* 7(2)**,** 99-109. doi: 10.1038/nrmicro2070.

Biedermann, T., Boettcher-Haberzeth, S., and Reichmann, E. (2013). Tissue engineering of skin for wound coverage. *Eur J Pediatr Surg* 23(5)**,** 375-382. doi: 10.1055/s-0033-1352529.

Bogoyevitch, M.A., and Kobe, B. (2006). Uses for JNK: the many and varied substrates of the c-Jun N-terminal kinases. *Microbiol Mol Biol Rev* 70(4)**,** 1061-1095. doi: 10.1128/MMBR.00025-06.

Boltjes, A., and van Wijk, F. (2014). Human dendritic cell functional specialization in steady-state and inflammation. *Front Immunol* 5**,** 131. doi: 10.3389/fimmu.2014.00131.

Bonham, K.S., and Kagan, J.C. (2014). Endosomes as platforms for NOD-like receptor signaling. *Cell Host Microbe* 15(5)**,** 523-525. doi: 10.1016/j.chom.2014.05.001.

Boraschi, D., and Tagliabue, A. (2013). The interleukin-1 receptor family. *Semin Immunol* 25(6)**,** 394-407. doi: 10.1016/j.smim.2013.10.023.

Bossaller, L., Chiang, P.I., Schmidt-Lauber, C., Ganesan, S., Kaiser, W.J., Rathinam, V.A., et al. (2012). Cutting edge: FAS (CD95) mediates noncanonical IL-1beta and IL-18 maturation via caspase-8 in an RIP3-independent manner. *J Immunol* 189(12)**,** 5508-5512. doi: 10.4049/jimmunol.1202121.

Brancho, D., Tanaka, N., Jaeschke, A., Ventura, J.J., Kelkar, N., Tanaka, Y., et al. (2003). Mechanism of p38 MAP kinase activation in vivo. *Genes Dev* 17(16)**,** 1969-1978. doi: 10.1101/gad.1107303.

Brenner, M., and Hearing, V.J. (2008). The protective role of melanin against UV damage in human skin. *Photochem Photobiol* 84(3)**,** 539-549. doi: 10.1111/j.1751-1097.2007.00226.x.

Brinkmann, V., Reichard, U., Goosmann, C., Fauler, B., Uhlemann, Y., Weiss, D.S., et al. (2004). Neutrophil extracellular traps kill bacteria. *Science* 303(5663)**,** 1532-1535. doi: 10.1126/science.1092385.

Brouns, S.J. (2012). Molecular biology. A Swiss army knife of immunity. *Science* 337(6096)**,** 808-809. doi: 10.1126/science.1227253.

Brown, G.D., Herre, J., Williams, D.L., Willment, J.A., Marshall, A.S., and Gordon, S. (2003). Dectin-1 mediates the biological effects of beta-glucans. *J Exp Med* 197(9)**,** 1119-1124. doi: 10.1084/jem.20021890.

Broz, P., and Dixit, V.M. (2016). Inflammasomes: mechanism of assembly, regulation and signalling. *Nat Rev Immunol* 16(7)**,** 407-420. doi: 10.1038/nri.2016.58.

Broz, P., and Monack, D.M. (2011). Molecular mechanisms of inflammasome activation during microbial infections. *Immunol Rev* 243(1)**,** 174-190. doi: 10.1111/j.1600-065X.2011.01041.x.

Broz, P., von Moltke, J., Jones, J.W., Vance, R.E., and Monack, D.M. (2010). Differential requirement for Caspase-1 autoproteolysis in pathogen-induced cell death and cytokine processing. *Cell Host Microbe* 8(6)**,** 471-483. doi: 10.1016/j.chom.2010.11.007.

Brubaker, S.W., Bonham, K.S., Zanoni, I., and Kagan, J.C. (2015). Innate immune pattern recognition: a cell biological perspective. *Annu Rev Immunol* 33**,** 257-290. doi: 10.1146/annurev-immunol-032414-112240.

Brydges, S.D., Broderick, L., McGeough, M.D., Pena, C.A., Mueller, J.L., and Hoffman, H.M. (2013). Divergence of IL-1, IL-18, and cell death in NLRP3 inflammasomopathies. *J Clin Invest* 123(11)**,** 4695-4705. doi: 10.1172/JCI71543.

Brydges, S.D., Mueller, J.L., McGeough, M.D., Pena, C.A., Misaghi, A., Gandhi, C., et al. (2009). Inflammasome-mediated disease animal models reveal roles for innate but not adaptive immunity. *Immunity* 30(6)**,** 875-887. doi: 10.1016/j.immuni.2009.05.005.

Burger, D., Chicheportiche, R., Giri, J.G., and Dayer, J.M. (1995). The inhibitory activity of human interleukin-1 receptor antagonist is enhanced by type II interleukin-1 soluble receptor and hindered by type I interleukin-1 soluble receptor. *J Clin Invest* 96(1)**,** 38-41. doi: 10.1172/JCI118045.

Campeau, E., Ruhl, V.E., Rodier, F., Smith, C.L., Rahmberg, B.L., Fuss, J.O., et al. (2009). A versatile viral system for expression and depletion of proteins in mammalian cells. *PLoS One* 4(8)**,** e6529. doi: 10.1371/journal.pone.0006529.

Canna, S.W., de Jesus, A.A., Gouni, S., Brooks, S.R., Marrero, B., Liu, Y., et al. (2014). An activating NLRC4 inflammasome mutation causes autoinflammation with recurrent macrophage activation syndrome. *Nat Genet* 46(10)**,** 1140-1146. doi: 10.1038/ng.3089.

Carbery, I.D., Ji, D., Harrington, A., Brown, V., Weinstein, E.J., Liaw, L., et al. (2010). Targeted genome modification in mice using zinc-finger nucleases. *Genetics* 186(2)**,** 451-459. doi: 10.1534/genetics.110.117002.

Carrasco, D., Stecher, M., Lefebvre, G.C., Logan, A.C., and Moy, R. (2015). An Open Label, Phase 2 Study of MABp1 Monotherapy for the Treatment of Acne Vulgaris and Psychiatric Comorbidity. *J Drugs Dermatol* 14(6)**,** 560-564.

Carthew, R.W., and Sontheimer, E.J. (2009). Origins and Mechanisms of miRNAs and siRNAs. *Cell* 136(4)**,** 642-655. doi: 10.1016/j.cell.2009.01.035.

Casar, B., Pinto, A., and Crespo, P. (2008). Essential role of ERK dimers in the activation of cytoplasmic but not nuclear substrates by ERK-scaffold complexes. *Mol Cell* 31(5)**,** 708-721. doi: 10.1016/j.molcel.2008.07.024.

Chae, J.J., Wood, G., Masters, S.L., Richard, K., Park, G., Smith, B.J., et al. (2006). The B30.2 domain of pyrin, the familial Mediterranean fever protein, interacts directly with caspase-1 to modulate IL-1beta production. *Proc Natl Acad Sci U S A* 103(26)**,** 9982-9987. doi: 10.1073/pnas.0602081103.

Chang, L., and Karin, M. (2001). Mammalian MAP kinase signalling cascades. *Nature* 410(6824)**,** 37-40. doi: 10.1038/35065000.

Charkoudian, N. (2010). Mechanisms and modifiers of reflex induced cutaneous vasodilation and vasoconstriction in humans. *J Appl Physiol (1985)* 109(4)**,** 1221-1228. doi: 10.1152/japplphysiol.00298.2010.

Chavarria-Smith, J., Mitchell, P.S., Ho, A.M., Daugherty, M.D., and Vance, R.E. (2016). Functional and Evolutionary Analyses Identify Proteolysis as a General Mechanism for NLRP1 Inflammasome Activation. *PLoS Pathog* 12(12)**,** e1006052. doi: 10.1371/journal.ppat.1006052.

Chen, C.J., Kono, H., Golenbock, D., Reed, G., Akira, S., and Rock, K.L. (2007). Identification of a key pathway required for the sterile inflammatory response triggered by dying cells. *Nat Med* 13(7)**,** 851-856. doi: 10.1038/nm1603.

Chen, C.J., Shi, Y., Hearn, A., Fitzgerald, K., Golenbock, D., Reed, G., et al. (2006). MyD88-dependent IL-1 receptor signaling is essential for gouty inflammation stimulated by monosodium urate crystals. *J Clin Invest* 116(8)**,** 2262-2271. doi: 10.1172/JCI28075.

Chen, K., Shanmugam, N.K., Pazos, M.A., Hurley, B.P., and Cherayil, B.J. (2016). Commensal Bacteria-Induced Inflammasome Activation in Mouse and Human Macrophages Is Dependent on Potassium Efflux but Does Not Require Phagocytosis or Bacterial Viability. *PLoS One* 11(8)**,** e0160937. doi: 10.1371/journal.pone.0160937.

Chen, K.W., Gross, C.J., Sotomayor, F.V., Stacey, K.J., Tschopp, J., Sweet, M.J., et al. (2014). The neutrophil NLRC4 inflammasome selectively promotes IL-1beta maturation without pyroptosis during acute Salmonella challenge. *Cell Rep* 8(2)**,** 570-582. doi: 10.1016/j.celrep.2014.06.028.

Chen, W., and Bowden, G.T. (1999). Activation of p38 MAP kinase and ERK are required for ultraviolet-B induced c-fos gene expression in human keratinocytes. *Oncogene* 18(52)**,** 7469-7476. doi: 10.1038/sj.onc.1203210.

Chen, W., Dong, Z., Valcic, S., Timmermann, B.N., and Bowden, G.T. (1999). Inhibition of ultraviolet B--induced c-fos gene expression and p38 mitogen-activated protein kinase activation by (-)-epigallocatechin gallate in a human keratinocyte cell line. *Mol Carcinog* 24(2)**,** 79-84.

Chen, W., Tang, Q., Gonzales, M.S., and Bowden, G.T. (2001). Role of p38 MAP kinases and ERK in mediating ultraviolet-B induced cyclooxygenase-2 gene expression in human keratinocytes. *Oncogene* 20(29)**,** 3921-3926. doi: 10.1038/sj.onc.1204530.

Chung, I.C., OuYang, C.N., Yuan, S.N., Li, H.P., Chen, J.T., Shieh, H.R., et al. (2016a). Pyk2 activates the NLRP3 inflammasome by directly phosphorylating ASC and contributes to inflammasome-dependent peritonitis. *Sci Rep* 6**,** 36214. doi: 10.1038/srep36214.

Chung, Y.H., Kim, D.H., and Lee, W.W. (2016b). Monosodium urate crystal-induced pro-interleukin-1beta production is post-transcriptionally regulated via the p38 signaling pathway in human monocytes. *Sci Rep* 6**,** 34533. doi: 10.1038/srep34533.

Cichorek, M., Wachulska, M., Stasiewicz, A., and Tyminska, A. (2013). Skin melanocytes: biology and development. *Postepy Dermatol Alergol* 30(1)**,** 30-41. doi: 10.5114/pdia.2013.33376.

Coll, R.C., Robertson, A.A., Chae, J.J., Higgins, S.C., Munoz-Planillo, R., Inserra, M.C., et al. (2015). A small-molecule inhibitor of the NLRP3 inflammasome for the treatment of inflammatory diseases. *Nat Med* 21(3)**,** 248-255. doi: 10.1038/nm.3806.

Contassot, E., Beer, H.D., and French, L.E. (2012). Interleukin-1, inflammasomes, autoinflammation and the skin. *Swiss Med Wkly* 142**,** w13590. doi: 10.4414/smw.2012.13590.

Coulthard, L.R., White, D.E., Jones, D.L., McDermott, M.F., and Burchill, S.A. (2009). p38(MAPK): stress responses from molecular mechanisms to therapeutics. *Trends Mol Med* 15(8)**,** 369-379. doi: 10.1016/j.molmed.2009.06.005.

Cuenda, A., and Rousseau, S. (2007). p38 MAP-kinases pathway regulation, function and role in human diseases. *Biochim Biophys Acta* 1773(8)**,** 1358-1375. doi: 10.1016/j.bbamcr.2007.03.010.

Cullen, B.R., Cherry, S., and tenOever, B.R. (2013). Is RNA interference a physiologically relevant innate antiviral immune response in mammals? *Cell Host Microbe* 14(4)**,** 374-378. doi: 10.1016/j.chom.2013.09.011.

Cummings, R.D., and McEver, R.P. (2009). "C-type Lectins," in *Essentials of Glycobiology,* eds. A. Varki, R.D. Cummings, J.D. Esko, H.H. Freeze, P. Stanley, C.R. Bertozzi, G.W. Hart & M.E. Etzler. 2nd ed (Cold Spring Harbor (NY)).

Czabotar, P.E., Lessene, G., Strasser, A., and Adams, J.M. (2014). Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy. *Nat Rev Mol Cell Biol* 15(1)**,** 49-63. doi: 10.1038/nrm3722.

da Silva Correia, J., Soldau, K., Christen, U., Tobias, P.S., and Ulevitch, R.J. (2001). Lipopolysaccharide is in close proximity to each of the proteins in its membrane receptor complex. transfer from CD14 to TLR4 and MD-2. *J Biol Chem* 276(24)**,** 21129-21135. doi: 10.1074/jbc.M009164200.

Dai, L., Aye Thu, C., Liu, X.Y., Xi, J., and Cheung, P.C. (2012). TAK1, more than just innate immunity. *IUBMB Life* 64(10)**,** 825-834. doi: 10.1002/iub.1078.

Dai, X., Tohyama, M., Murakami, M., and Sayama, K. (2017). Epidermal keratinocytes sense dsRNA via the NLRP3 inflammasome, mediating interleukin (IL)-1beta and IL-18 release. *Exp Dermatol*. doi: 10.1111/exd.13334.

de Almeida, L., Khare, S., Misharin, A.V., Patel, R., Ratsimandresey, R.A., Wallin, M.C., et al. (2015). The PYRIN Domain-only Protein POP1 Inhibits Inflammasome Assembly and Ameliorates Inflammatory Disease. *Immunity* 43(2)**,** 264-276. doi: 10.1016/j.immuni.2015.07.018.

De Chiara, G., Marcocci, M.E., Torcia, M., Lucibello, M., Rosini, P., Bonini, P., et al. (2006). Bcl-2 Phosphorylation by p38 MAPK: identification of target sites and biologic consequences. *J Biol Chem* 281(30)**,** 21353-21361. doi: 10.1074/jbc.M511052200.

Denes, A., Coutts, G., Lenart, N., Cruickshank, S.M., Pelegrin, P., Skinner, J., et al. (2015). AIM2 and NLRC4 inflammasomes contribute with ASC to acute brain injury independently of NLRP3. *Proc Natl Acad Sci U S A* 112(13)**,** 4050-4055. doi: 10.1073/pnas.1419090112.

Derijard, B., Raingeaud, J., Barrett, T., Wu, I.H., Han, J., Ulevitch, R.J., et al. (1995). Independent human MAP-kinase signal transduction pathways defined by MEK and MKK isoforms. *Science* 267(5198)**,** 682-685.

Dhillon, A.S., Hagan, S., Rath, O., and Kolch, W. (2007). MAP kinase signalling pathways in cancer. *Oncogene* 26(22)**,** 3279-3290. doi: 10.1038/sj.onc.1210421.

Di Paolo, N.C., and Shayakhmetov, D.M. (2016). Interleukin 1alpha and the inflammatory process. *Nat Immunol* 17(8)**,** 906-913. doi: 10.1038/ni.3503.

Dinarello, C.A. (1996). Biologic basis for interleukin-1 in disease. *Blood* 87(6)**,** 2095-2147.

Dinarello, C.A. (2009). Immunological and inflammatory functions of the interleukin-1 family. *Annu Rev Immunol* 27**,** 519-550. doi: 10.1146/annurev.immunol.021908.132612.

Dinarello, C.A. (2011). Blocking interleukin-1beta in acute and chronic autoinflammatory diseases. *J Intern Med* 269(1)**,** 16-28. doi: 10.1111/j.1365-2796.2010.02313.x.

Dinarello, C.A., Goldin, N.P., and Wolff, S.M. (1974). Demonstration and characterization of two distinct human leukocytic pyrogens. *J Exp Med* 139(6)**,** 1369-1381.

Dinarello, C.A., Novick, D., Kim, S., and Kaplanski, G. (2013). Interleukin-18 and IL-18 binding protein. *Front Immunol* 4**,** 289. doi: 10.3389/fimmu.2013.00289.

Dinarello, C.A., Renfer, L., and Wolff, S.M. (1977). Human leukocytic pyrogen: purification and development of a radioimmunoassay. *Proc Natl Acad Sci U S A* 74(10)**,** 4624-4627.

Ding, J., Wang, K., Liu, W., She, Y., Sun, Q., Shi, J., et al. (2016). Pore-forming activity and structural autoinhibition of the gasdermin family. *Nature* 535(7610)**,** 111-116. doi: 10.1038/nature18590.

Ding, S.W. (2010). RNA-based antiviral immunity. *Nat Rev Immunol* 10(9)**,** 632-644. doi: 10.1038/nri2824.

Dombrowski, Y., Peric, M., Koglin, S., Kammerbauer, C., Goss, C., Anz, D., et al. (2011). Cytosolic DNA triggers inflammasome activation in keratinocytes in psoriatic lesions. *Sci Transl Med* 3(82)**,** 82ra38. doi: 10.1126/scitranslmed.3002001.

Dombrowski, Y., Peric, M., Koglin, S., Kaymakanov, N., Schmezer, V., Reinholz, M., et al. (2012). Honey bee (Apis mellifera) venom induces AIM2 inflammasome activation in human keratinocytes. *Allergy* 67(11)**,** 1400-1407. doi: 10.1111/all.12022.

Dong, C., Yang, D.D., Wysk, M., Whitmarsh, A.J., Davis, R.J., and Flavell, R.A. (1998). Defective T cell differentiation in the absence of Jnk1. *Science* 282(5396)**,** 2092-2095.

Dorfleutner, A., Chu, L., and Stehlik, C. (2015). Inhibiting the inflammasome: one domain at a time. *Immunol Rev* 265(1)**,** 205-216. doi: 10.1111/imr.12290.

Dorfleutner, A., Talbott, S.J., Bryan, N.B., Funya, K.N., Rellick, S.L., Reed, J.C., et al. (2007). A Shope Fibroma virus PYRIN-only protein modulates the host immune response. *Virus Genes* 35(3)**,** 685-694. doi: 10.1007/s11262-007-0141-9.

Dostert, C., Petrilli, V., Van Bruggen, R., Steele, C., Mossman, B.T., and Tschopp, J. (2008). Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. *Science* 320(5876)**,** 674-677. doi: 10.1126/science.1156995.

Duesbery, N.S., Webb, C.P., Leppla, S.H., Gordon, V.M., Klimpel, K.R., Copeland, T.D., et al. (1998). Proteolytic inactivation of MAP-kinase-kinase by anthrax lethal factor. *Science* 280(5364)**,** 734-737.

Duewell, P., Kono, H., Rayner, K.J., Sirois, C.M., Vladimer, G., Bauernfeind, F.G., et al. (2010). NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature* 464(7293)**,** 1357-1361. doi: 10.1038/nature08938.

Dunkelberger, J.R., and Song, W.C. (2010). Complement and its role in innate and adaptive immune responses. *Cell Res* 20(1)**,** 34-50. doi: 10.1038/cr.2009.139.

Efimova, T., Broome, A.M., and Eckert, R.L. (2003). A regulatory role for p38 delta MAPK in keratinocyte differentiation. Evidence for p38 delta-ERK1/2 complex formation. *J Biol Chem* 278(36)**,** 34277-34285. doi: 10.1074/jbc.M302759200.

Elmore, S. (2007). Apoptosis: a review of programmed cell death. *Toxicol Pathol* 35(4)**,** 495-516. doi: 10.1080/01926230701320337.

Enslen, H., Raingeaud, J., and Davis, R.J. (1998). Selective activation of p38 mitogen-activated protein (MAP) kinase isoforms by the MAP kinase kinases MKK3 and MKK6. *J Biol Chem* 273(3)**,** 1741-1748.

Erwig, L.P., and Henson, P.M. (2008). Clearance of apoptotic cells by phagocytes. *Cell Death Differ* 15(2)**,** 243-250. doi: 10.1038/sj.cdd.4402184.

Fan, L., Busser, B.W., Lifsted, T.Q., Oukka, M., Lo, D., and Laufer, T.M. (2003). Antigen presentation by keratinocytes directs autoimmune skin disease. *Proc Natl Acad Sci U S A* 100(6)**,** 3386-3391. doi: 10.1073/pnas.0437899100.

Faustin, B., Lartigue, L., Bruey, J.M., Luciano, F., Sergienko, E., Bailly-Maitre, B., et al. (2007). Reconstituted NALP1 inflammasome reveals two-step mechanism of caspase-1 activation. *Mol Cell* 25(5)**,** 713-724. doi: 10.1016/j.molcel.2007.01.032.

Feldmeyer, L., Keller, M., Niklaus, G., Hohl, D., Werner, S., and Beer, H.D. (2007). The inflammasome mediates UVB-induced activation and secretion of interleukin-1beta by keratinocytes. *Curr Biol* 17(13)**,** 1140-1145. doi: 10.1016/j.cub.2007.05.074.

Feldmeyer, L., Werner, S., French, L.E., and Beer, H.D. (2010). Interleukin-1, inflammasomes and the skin. *Eur J Cell Biol* 89(9)**,** 638-644. doi: 10.1016/j.ejcb.2010.04.008.

Feng, L., Zhou, X., Liao, J., and Omary, M.B. (1999). Pervanadate-mediated tyrosine phosphorylation of keratins 8 and 19 via a p38 mitogen-activated protein kinase-dependent pathway. *J Cell Sci* 112 (Pt 13)**,** 2081-2090.

Fenini, G., Contassot, E., and French, L.E. (2017). Potential of IL-1, IL-18 and Inflammasome Inhibition for the Treatment of Inflammatory Skin Diseases. *Frontiers in Pharmacology* 8(278). doi: 10.3389/fphar.2017.00278.

Fettelschoss, A., Kistowska, M., LeibundGut-Landmann, S., Beer, H.D., Johansen, P., Senti, G., et al. (2011). Inflammasome activation and IL-1beta target IL-1alpha for secretion as opposed to surface expression. *Proc Natl Acad Sci U S A* 108(44)**,** 18055-18060. doi: 10.1073/pnas.1109176108.

Fink, S.L., and Cookson, B.T. (2005). Apoptosis, pyroptosis, and necrosis: mechanistic description of dead and dying eukaryotic cells. *Infect Immun* 73(4)**,** 1907-1916. doi: 10.1128/IAI.73.4.1907-1916.2005.

French, F.M.F.C. (1997). A candidate gene for familial Mediterranean fever. *Nat Genet* 17(1)**,** 25-31. doi: 10.1038/ng0997-25.

Gaestel, M. (2006). MAPKAP kinases - MKs - two's company, three's a crowd. *Nat Rev Mol Cell Biol* 7(2)**,** 120-130. doi: 10.1038/nrm1834.

Gaitanis, G., Magiatis, P., Hantschke, M., Bassukas, I.D., and Velegraki, A. (2012). The Malassezia genus in skin and systemic diseases. *Clin Microbiol Rev* 25(1)**,** 106-141. doi: 10.1128/CMR.00021-11.

Gaj, T., Gersbach, C.A., and Barbas, C.F., 3rd (2013). ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. *Trends Biotechnol* 31(7)**,** 397-405. doi: 10.1016/j.tibtech.2013.04.004.

Gallo, R.L. (2017). Human Skin Is the Largest Epithelial Surface for Interaction with Microbes. *J Invest Dermatol* 137(6)**,** 1213-1214. doi: 10.1016/j.jid.2016.11.045.

Gay, N.J., and Gangloff, M. (2007). Structure and function of Toll receptors and their ligands. *Annu Rev Biochem* 76**,** 141-165. doi: 10.1146/annurev.biochem.76.060305.151318.

Gebhardt, T., Wakim, L.M., Eidsmo, L., Reading, P.C., Heath, W.R., and Carbone, F.R. (2009). Memory T cells in nonlymphoid tissue that provide enhanced local immunity during infection with herpes simplex virus. *Nat Immunol* 10(5)**,** 524-530. doi: 10.1038/ni.1718.

Geijtenbeek, T.B., and Gringhuis, S.I. (2009). Signalling through C-type lectin receptors: shaping immune responses. *Nat Rev Immunol* 9(7)**,** 465-479. doi: 10.1038/nri2569.

Gery, I., Gershon, R.K., and Waksman, B.H. (1972). Potentiation of the T-lymphocyte response to mitogens. I. The responding cell. *J Exp Med* 136(1)**,** 128-142.

Ghosn, E.E., Cassado, A.A., Govoni, G.R., Fukuhara, T., Yang, Y., Monack, D.M., et al. (2010). Two physically, functionally, and developmentally distinct peritoneal macrophage subsets. *Proc Natl Acad Sci U S A* 107(6)**,** 2568-2573. doi: 10.1073/pnas.0915000107.

Girardin, S.E., Boneca, I.G., Viala, J., Chamaillard, M., Labigne, A., Thomas, G., et al. (2003). Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. *J Biol Chem* 278(11)**,** 8869-8872. doi: 10.1074/jbc.C200651200.

Goblos, A., Danis, J., Vas, K., Bata-Csorgo, Z., Kemeny, L., and Szell, M. (2016). Keratinocytes express functional CARD18, a negative regulator of inflammasome activation, and its altered expression in psoriasis may contribute to disease pathogenesis. *Mol Immunol* 73**,** 10-18. doi: 10.1016/j.molimm.2016.03.009.

Goldbach-Mansky, R. (2011). Current status of understanding the pathogenesis and management of patients with NOMID/CINCA. *Curr Rheumatol Rep* 13(2)**,** 123-131. doi: 10.1007/s11926-011-0165-y.

Gomez del Arco, P., Martinez-Martinez, S., Maldonado, J.L., Ortega-Perez, I., and Redondo, J.M. (2000). A role for the p38 MAP kinase pathway in the nuclear shuttling of NFATp. *J Biol Chem* 275(18)**,** 13872-13878.

Gringhuis, S.I., Kaptein, T.M., Wevers, B.A., Theelen, B., van der Vlist, M., Boekhout, T., et al. (2012). Dectin-1 is an extracellular pathogen sensor for the induction and processing of IL-1beta via a noncanonical caspase-8 inflammasome. *Nat Immunol* 13(3)**,** 246-254. doi: 10.1038/ni.2222.

Gross, C.J., Mishra, R., Schneider, K.S., Medard, G., Wettmarshausen, J., Dittlein, D.C., et al. (2016). K+ Efflux-Independent NLRP3 Inflammasome Activation by Small Molecules Targeting Mitochondria. *Immunity* 45(4)**,** 761-773. doi: 10.1016/j.immuni.2016.08.010.

Gross, O., Poeck, H., Bscheider, M., Dostert, C., Hannesschlager, N., Endres, S., et al. (2009). Syk kinase signalling couples to the Nlrp3 inflammasome for anti-fungal host defence. *Nature* 459(7245)**,** 433-436. doi: 10.1038/nature07965.

Gross, O., Yazdi, A.S., Thomas, C.J., Masin, M., Heinz, L.X., Guarda, G., et al. (2012). Inflammasome activators induce interleukin-1alpha secretion via distinct pathways with differential requirement for the protease function of caspase-1. *Immunity* 36(3)**,** 388-400. doi: 10.1016/j.immuni.2012.01.018.

Guarda, G., Zenger, M., Yazdi, A.S., Schroder, K., Ferrero, I., Menu, P., et al. (2011). Differential expression of NLRP3 among hematopoietic cells. *J Immunol* 186(4)**,** 2529-2534. doi: 10.4049/jimmunol.1002720.

Guma, M., Ronacher, L., Liu-Bryan, R., Takai, S., Karin, M., and Corr, M. (2009). Caspase 1-independent activation of interleukin-1beta in neutrophil-predominant inflammation. *Arthritis Rheum* 60(12)**,** 3642-3650. doi: 10.1002/art.24959.

Haas, D., and Hoffmann, G.F. (2006). Mevalonate kinase deficiencies: from mevalonic aciduria to hyperimmunoglobulinemia D syndrome. *Orphanet J Rare Dis* 1**,** 13. doi: 10.1186/1750-1172-1-13.

Halle, A., Hornung, V., Petzold, G.C., Stewart, C.R., Monks, B.G., Reinheckel, T., et al. (2008). The NALP3 inflammasome is involved in the innate immune response to amyloid-beta. *Nat Immunol* 9(8)**,** 857-865. doi: 10.1038/ni.1636.

Han, J., Lee, J.D., Tobias, P.S., and Ulevitch, R.J. (1993). Endotoxin induces rapid protein tyrosine phosphorylation in 70Z/3 cells expressing CD14. *J Biol Chem* 268(33)**,** 25009-25014.

Hara, H., Tsuchiya, K., Kawamura, I., Fang, R., Hernandez-Cuellar, E., Shen, Y., et al. (2013). Phosphorylation of the adaptor ASC acts as a molecular switch that controls the formation of speck-like aggregates and inflammasome activity. *Nat Immunol* 14(12)**,** 1247-1255. doi: 10.1038/ni.2749.

Hau, C.S., Kanda, N., Noda, S., Tatsuta, A., Kamata, M., Shibata, S., et al. (2013). Visfatin enhances the production of cathelicidin antimicrobial peptide, human beta-defensin-2, human beta-defensin-3, and S100A7 in human keratinocytes and their orthologs in murine imiquimod-induced psoriatic skin. *Am J Pathol* 182(5)**,** 1705-1717. doi: 10.1016/j.ajpath.2013.01.044.

Hayashi, F., Smith, K.D., Ozinsky, A., Hawn, T.R., Yi, E.C., Goodlett, D.R., et al. (2001). The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature* 410(6832)**,** 1099-1103. doi: 10.1038/35074106.

Hazzalin, C.A., Cano, E., Cuenda, A., Barratt, M.J., Cohen, P., and Mahadevan, L.C. (1996). p38/RK is essential for stress-induced nuclear responses: JNK/SAPKs and c-Jun/ATF-2 phosphorylation are insufficient. *Curr Biol* 6(8)**,** 1028-1031.

He, W.T., Wan, H., Hu, L., Chen, P., Wang, X., Huang, Z., et al. (2015). Gasdermin D is an executor of pyroptosis and required for interleukin-1beta secretion. *Cell Res* 25(12)**,** 1285-1298. doi: 10.1038/cr.2015.139.

He, Y., Zeng, M.Y., Yang, D., Motro, B., and Nunez, G. (2016). NEK7 is an essential mediator of NLRP3 activation downstream of potassium efflux. *Nature* 530(7590)**,** 354-357. doi: 10.1038/nature16959.

Hernandez-Cuellar, E., Tsuchiya, K., Hara, H., Fang, R., Sakai, S., Kawamura, I., et al. (2012). Cutting edge: nitric oxide inhibits the NLRP3 inflammasome. *J Immunol* 189(11)**,** 5113-5117. doi: 10.4049/jimmunol.1202479.

Hernandez-Pigeon, H., Jean, C., Charruyer, A., Haure, M.J., Titeux, M., Tonasso, L., et al. (2006). Human keratinocytes acquire cellular cytotoxicity under UV-B irradiation. Implication of granzyme B and perforin. *J Biol Chem* 281(19)**,** 13525-13532. doi: 10.1074/jbc.M512694200.

Hise, A.G., Tomalka, J., Ganesan, S., Patel, K., Hall, B.A., Brown, G.D., et al. (2009). An essential role for the NLRP3 inflammasome in host defense against the human fungal pathogen Candida albicans. *Cell Host Microbe* 5(5)**,** 487-497. doi: 10.1016/j.chom.2009.05.002.

Hoebe, K., Georgel, P., Rutschmann, S., Du, X., Mudd, S., Crozat, K., et al. (2005). CD36 is a sensor of diacylglycerides. *Nature* 433(7025)**,** 523-527. doi: 10.1038/nature03253.

Hoffman, H.M., Mueller, J.L., Broide, D.H., Wanderer, A.A., and Kolodner, R.D. (2001). Mutation of a new gene encoding a putative pyrin-like protein causes familial cold autoinflammatory syndrome and Muckle-Wells syndrome. *Nat Genet* 29(3)**,** 301-305. doi: 10.1038/ng756.

Hornung, V., Bauernfeind, F., Halle, A., Samstad, E.O., Kono, H., Rock, K.L., et al. (2008). Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization. *Nat Immunol* 9(8)**,** 847-856. doi: 10.1038/ni.1631.

Hoss, F., Rodriguez-Alcazar, J.F., and Latz, E. (2017). Assembly and regulation of ASC specks. *Cell Mol Life Sci* 74(7)**,** 1211-1229. doi: 10.1007/s00018-016-2396-6.

Hsu, L.C., Ali, S.R., McGillivray, S., Tseng, P.H., Mariathasan, S., Humke, E.W., et al. (2008). A NOD2-NALP1 complex mediates caspase-1-dependent IL-1beta secretion in response to Bacillus anthracis infection and muramyl dipeptide. *Proc Natl Acad Sci U S A* 105(22)**,** 7803-7808. doi: 10.1073/pnas.0802726105.

Hua, K.F., Chou, J.C., Ka, S.M., Tasi, Y.L., Chen, A., Wu, S.H., et al. (2015). Cyclooxygenase-2 regulates NLRP3 inflammasome-derived IL-1beta production. *J Cell Physiol* 230(4)**,** 863-874. doi: 10.1002/jcp.24815.

Huang, C., Ma, W.Y., Maxiner, A., Sun, Y., and Dong, Z. (1999). p38 kinase mediates UV-induced phosphorylation of p53 protein at serine 389. *J Biol Chem* 274(18)**,** 12229-12235.

Hull, K.M., Drewe, E., Aksentijevich, I., Singh, H.K., Wong, K., McDermott, E.M., et al. (2002). The TNF receptor-associated periodic syndrome (TRAPS): emerging concepts of an autoinflammatory disorder. *Medicine (Baltimore)* 81(5)**,** 349-368.

Huot, J., Houle, F., Marceau, F., and Landry, J. (1997). Oxidative stress-induced actin reorganization mediated by the p38 mitogen-activated protein kinase/heat shock protein 27 pathway in vascular endothelial cells. *Circ Res* 80(3)**,** 383-392.

Imhof, L., Meier, B., Frei, P., Kamarachev, J., Rogler, G., Kolios, A., et al. (2015). Severe Sweet's Syndrome with Elevated Cutaneous Interleukin-1beta after Azathioprine Exposure: Case Report and Review of the Literature. *Dermatology* 230(4)**,** 293-298. doi: 10.1159/000371879.

Infevers (2017). *Infevers: an online database for autoinflammatory mutations. Copyright.* [Online]. http://fmf.igh.cnrs.fr/ISSAID/infevers/. [Accessed March 20, 2017].

Ishitani, T., Kishida, S., Hyodo-Miura, J., Ueno, N., Yasuda, J., Waterman, M., et al. (2003). The TAK1-NLK mitogen-activated protein kinase cascade functions in the Wnt-5a/Ca(2+) pathway to antagonize Wnt/beta-catenin signaling. *Mol Cell Biol* 23(1)**,** 131-139.

Ito, M., Shichita, T., Okada, M., Komine, R., Noguchi, Y., Yoshimura, A., et al. (2015). Bruton's tyrosine kinase is essential for NLRP3 inflammasome activation and contributes to ischaemic brain injury. *Nat Commun* 6**,** 7360. doi: 10.1038/ncomms8360.

Janeway, C.A., Jr. (1989). Approaching the asymptote? Evolution and revolution in immunology. *Cold Spring Harb Symp Quant Biol* 54 Pt 1**,** 1-13.

Jeru, I., Duquesnoy, P., Fernandes-Alnemri, T., Cochet, E., Yu, J.W., Lackmy-Port-Lis, M., et al. (2008). Mutations in NALP12 cause hereditary periodic fever syndromes. *Proc Natl Acad Sci U S A* 105(5)**,** 1614-1619. doi: 10.1073/pnas.0708616105.

Jeru, I., Papin, S., L'Hoste, S., Duquesnoy, P., Cazeneuve, C., Camonis, J., et al. (2005). Interaction of pyrin with 14.3.3 in an isoform-specific and phosphorylation-dependent manner regulates its translocation to the nucleus. *Arthritis Rheum* 52(6)**,** 1848-1857. doi: 10.1002/art.21050.

Jesus, A.A., and Goldbach-Mansky, R. (2014). IL-1 blockade in autoinflammatory syndromes. *Annu Rev Med* 65**,** 223-244. doi: 10.1146/annurev-med-061512-150641.

Jiang, Y., Gram, H., Zhao, M., New, L., Gu, J., Feng, L., et al. (1997). Characterization of the structure and function of the fourth member of p38 group mitogen-activated protein kinases, p38delta. *J Biol Chem* 272(48)**,** 30122-30128.

Jin, Y., Mailloux, C.M., Gowan, K., Riccardi, S.L., LaBerge, G., Bennett, D.C., et al. (2007). NALP1 in vitiligo-associated multiple autoimmune disease. *N Engl J Med* 356(12)**,** 1216-1225. doi: 10.1056/NEJMoa061592.

Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J.A., and Charpentier, E. (2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* 337(6096)**,** 816-821. doi: 10.1126/science.1225829.

Johansen, C., Kragballe, K., Westergaard, M., Henningsen, J., Kristiansen, K., and Iversen, L. (2005). The mitogen-activated protein kinases p38 and ERK1/2 are increased in lesional psoriatic skin. *Br J Dermatol* 152(1)**,** 37-42. doi: 10.1111/j.1365-2133.2004.06304.x.

Johnson, D.B., and Puzanov, I. (2015). Treatment of NRAS-mutant melanoma. *Curr Treat Options Oncol* 16(4)**,** 15. doi: 10.1007/s11864-015-0330-z.

Johnston, J.B., Barrett, J.W., Nazarian, S.H., Goodwin, M., Ricciuto, D., Wang, G., et al. (2005). A poxvirus-encoded pyrin domain protein interacts with ASC-1 to inhibit host inflammatory and apoptotic responses to infection. *Immunity* 23(6)**,** 587-598. doi: 10.1016/j.immuni.2005.10.003.

Jorgensen, I., and Miao, E.A. (2015). Pyroptotic cell death defends against intracellular pathogens. *Immunol Rev* 265(1)**,** 130-142. doi: 10.1111/imr.12287.

Jorgensen, I., Rayamajhi, M., and Miao, E.A. (2017). Programmed cell death as a defence against infection. *Nat Rev Immunol* 17(3)**,** 151-164. doi: 10.1038/nri.2016.147.

Jubin, K., Martin, Y., Lawrence-Watt, D.J., and Sharpe, J.R. (2011). A fully autologous co-culture system utilising non-irradiated autologous fibroblasts to support the expansion of human keratinocytes for clinical use. *Cytotechnology* 63(6)**,** 655-662. doi: 10.1007/s10616-011-9382-5.

Juliana, C., Fernandes-Alnemri, T., Kang, S., Farias, A., Qin, F., and Alnemri, E.S. (2012). Non-transcriptional priming and deubiquitination regulate NLRP3 inflammasome activation. *J Biol Chem* 287(43)**,** 36617-36622. doi: 10.1074/jbc.M112.407130.

Kailasan Vanaja, S., Rathinam, V.A., Atianand, M.K., Kalantari, P., Skehan, B., Fitzgerald, K.A., et al. (2014). Bacterial RNA:DNA hybrids are activators of the NLRP3 inflammasome. *Proc Natl Acad Sci U S A* 111(21)**,** 7765-7770. doi: 10.1073/pnas.1400075111.

Kalali, B.N., Kollisch, G., Mages, J., Muller, T., Bauer, S., Wagner, H., et al. (2008). Double-stranded RNA induces an antiviral defense status in epidermal keratinocytes through TLR3-, PKR-, and MDA5/RIG-I-mediated differential signaling. *J Immunol* 181(4)**,** 2694-2704.

Kamada, N., Chen, G.Y., Inohara, N., and Nunez, G. (2013). Control of pathogens and pathobionts by the gut microbiota. *Nat Immunol* 14(7)**,** 685-690. doi: 10.1038/ni.2608.

Karre, K. (2002). NK cells, MHC class I molecules and the missing self. *Scand J Immunol* 55(3)**,** 221-228.

Kashem, S.W., Haniffa, M., and Kaplan, D.H. (2017). Antigen-Presenting Cells in the Skin. *Annu Rev Immunol*. doi: 10.1146/annurev-immunol-051116-052215.

Kato, H., Takeuchi, O., Mikamo-Satoh, E., Hirai, R., Kawai, T., Matsushita, K., et al. (2008). Length-dependent recognition of double-stranded ribonucleic acids by retinoic acid-inducible gene-I and melanoma differentiation-associated gene 5. *J Exp Med* 205(7)**,** 1601-1610. doi: 10.1084/jem.20080091.

Kayagaki, N., Stowe, I.B., Lee, B.L., O'Rourke, K., Anderson, K., Warming, S., et al. (2015). Caspase-11 cleaves gasdermin D for non-canonical inflammasome signalling. *Nature* 526(7575)**,** 666-671. doi: 10.1038/nature15541.

Kayagaki, N., Warming, S., Lamkanfi, M., Vande Walle, L., Louie, S., Dong, J., et al. (2011). Non-canonical inflammasome activation targets caspase-11. *Nature* 479(7371)**,** 117-121. doi: 10.1038/nature10558.

Kedzierski, L., Montgomery, J., Curtis, J., and Handman, E. (2004). Leucine-rich repeats in host-pathogen interactions. *Arch Immunol Ther Exp (Warsz)* 52(2)**,** 104-112.

Keller, M., Ruegg, A., Werner, S., and Beer, H.D. (2008). Active caspase-1 is a regulator of unconventional protein secretion. *Cell* 132(5)**,** 818-831. doi: 10.1016/j.cell.2007.12.040.

Kerur, N., Veettil, M.V., Sharma-Walia, N., Bottero, V., Sadagopan, S., Otageri, P., et al. (2011). IFI16 acts as a nuclear pathogen sensor to induce the inflammasome in response to Kaposi Sarcoma-

associated herpesvirus infection. *Cell Host Microbe* 9(5), 363-375. doi: 10.1016/j.chom.2011.04.008.
Khare, S., Ratsimandresey, R.A., de Almeida, L., Cuda, C.M., Rellick, S.L., Misharin, A.V., et al. (2014). The PYRIN domain-only protein POP3 inhibits ALR inflammasomes and regulates responses to infection with DNA viruses. *Nat Immunol* 15(4), 343-353. doi: 10.1038/ni.2829.
Kim, A.L., Labasi, J.M., Zhu, Y., Tang, X., McClure, K., Gabel, C.A., et al. (2005). Role of p38 MAPK in UVB-induced inflammatory responses in the skin of SKH-1 hairless mice. *J Invest Dermatol* 124(6), 1318-1325. doi: 10.1111/j.0022-202X.2005.23747.x.
Kim, B., Lee, Y., Kim, E., Kwak, A., Ryoo, S., Bae, S.H., et al. (2013). The Interleukin-1alpha Precursor is Biologically Active and is Likely a Key Alarmin in the IL-1 Family of Cytokines. *Front Immunol* 4, 391. doi: 10.3389/fimmu.2013.00391.
Kim, B.J., Ryu, S.W., and Song, B.J. (2006). JNK- and p38 kinase-mediated phosphorylation of Bax leads to its activation and mitochondrial translocation and to apoptosis of human hepatoma HepG2 cells. *J Biol Chem* 281(30), 21256-21265. doi: 10.1074/jbc.M510644200.
Kim, B.S. (2015). Innate lymphoid cells in the skin. *J Invest Dermatol* 135(3), 673-678. doi: 10.1038/jid.2014.401.
Kim, B.S., Miyagawa, F., Cho, Y.H., Bennett, C.L., Clausen, B.E., and Katz, S.I. (2009). Keratinocytes function as accessory cells for presentation of endogenous antigen expressed in the epidermis. *J Invest Dermatol* 129(12), 2805-2817. doi: 10.1038/jid.2009.176.
Kim, C., Sano, Y., Todorova, K., Carlson, B.A., Arpa, L., Celada, A., et al. (2008). The kinase p38 alpha serves cell type-specific inflammatory functions in skin injury and coordinates pro- and anti-inflammatory gene expression. *Nat Immunol* 9(9), 1019-1027. doi: 10.1038/ni.1640.
Kim, H.S., Shin, T.H., Yang, S.R., Seo, M.S., Kim, D.J., Kang, S.K., et al. (2010). Implication of NOD1 and NOD2 for the differentiation of multipotent mesenchymal stem cells derived from human umbilical cord blood. *PLoS One* 5(10), e15369. doi: 10.1371/journal.pone.0015369.
Kimura, T., Jain, A., Choi, S.W., Mandell, M.A., Schroder, K., Johansen, T., et al. (2015). TRIM-mediated precision autophagy targets cytoplasmic regulators of innate immunity. *J Cell Biol* 210(6), 973-989. doi: 10.1083/jcb.201503023.
Kinoshita, E., Kinoshita-Kikuta, E., and Koike, T. (2009). Separation and detection of large phosphoproteins using Phos-tag SDS-PAGE. *Nat Protoc* 4(10), 1513-1521. doi: 10.1038/nprot.2009.154.
Kistowska, M., Fenini, G., Jankovic, D., Feldmeyer, L., Kerl, K., Bosshard, P., et al. (2014a). Malassezia yeasts activate the NLRP3 inflammasome in antigen-presenting cells via Syk-kinase signalling. *Exp Dermatol* 23(12), 884-889. doi: 10.1111/exd.12552.
Kistowska, M., Gehrke, S., Jankovic, D., Kerl, K., Fettelschoss, A., Feldmeyer, L., et al. (2014b). IL-1beta drives inflammatory responses to propionibacterium acnes in vitro and in vivo. *J Invest Dermatol* 134(3), 677-685. doi: 10.1038/jid.2013.438.
Kitada, K., Ishishita, S., Tosaka, K., Takahashi, R., Ueda, M., Keng, V.W., et al. (2007). Transposon-tagged mutagenesis in the rat. *Nat Methods* 4(2), 131-133. doi: 10.1038/nmeth1002.
Kitamura, A., Sasaki, Y., Abe, T., Kano, H., and Yasutomo, K. (2014). An inherited mutation in NLRC4 causes autoinflammation in human and mice. *J Exp Med* 211(12), 2385-2396. doi: 10.1084/jem.20141091.
Kleinstiver, B.P., Prew, M.S., Tsai, S.Q., Topkar, V.V., Nguyen, N.T., Zheng, Z., et al. (2015). Engineered CRISPR-Cas9 nucleases with altered PAM specificities. *Nature* 523(7561), 481-485. doi: 10.1038/nature14592.
Klose, C.S., and Artis, D. (2016). Innate lymphoid cells as regulators of immunity, inflammation and tissue homeostasis. *Nat Immunol* 17(7), 765-774. doi: 10.1038/ni.3489.
Klug, A. (2010). The discovery of zinc fingers and their applications in gene regulation and genome manipulation. *Annu Rev Biochem* 79, 213-231. doi: 10.1146/annurev-biochem-010909-095056.
Kolios, A.G., Maul, J.T., Meier, B., Kerl, K., Traidl-Hoffmann, C., Hertl, M., et al. (2015). Canakinumab in adults with steroid-refractory pyoderma gangrenosum. *Br J Dermatol* 173(5), 1216-1223. doi: 10.1111/bjd.14037.
Kortmann, J., Brubaker, S.W., and Monack, D.M. (2015). Cutting Edge: Inflammasome Activation in Primary Human Macrophages Is Dependent on Flagellin. *J Immunol* 195(3), 815-819. doi: 10.4049/jimmunol.1403100.

Kovarova, M., Hesker, P.R., Jania, L., Nguyen, M., Snouwaert, J.N., Xiang, Z., et al. (2012). NLRP1-dependent pyroptosis leads to acute lung injury and morbidity in mice. *J Immunol* 189(4)**,** 2006-2016. doi: 10.4049/jimmunol.1201065.

Kowalczyk, A.P., and Green, K.J. (2013). Structure, function, and regulation of desmosomes. *Prog Mol Biol Transl Sci* 116**,** 95-118. doi: 10.1016/B978-0-12-394311-8.00005-4.

Krachler, A.M., Woolery, A.R., and Orth, K. (2011). Manipulation of kinase signaling by bacterial pathogens. *J Cell Biol* 195(7)**,** 1083-1092. doi: 10.1083/jcb.201107132.

Kruger, P., Saffarzadeh, M., Weber, A.N., Rieber, N., Radsak, M., von Bernuth, H., et al. (2015). Neutrophils: Between host defence, immune modulation, and tissue injury. *PLoS Pathog* 11(3)**,** e1004651. doi: 10.1371/journal.ppat.1004651.

Kumar, S., Boehm, J., and Lee, J.C. (2003). p38 MAP kinases: key signalling molecules as therapeutic targets for inflammatory diseases. *Nat Rev Drug Discov* 2(9)**,** 717-726. doi: 10.1038/nrd1177.

Kyriakis, J.M., and Avruch, J. (2012). Mammalian MAPK signal transduction pathways activated by stress and inflammation: a 10-year update. *Physiol Rev* 92(2)**,** 689-737. doi: 10.1152/physrev.00028.2011.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227(5259)**,** 680-685.

Latz, E., Xiao, T.S., and Stutz, A. (2013). Activation and regulation of the inflammasomes. *Nat Rev Immunol* 13(6)**,** 397-411. doi: 10.1038/nri3452.

Lebre, M.C., van der Aar, A.M., van Baarsen, L., van Capel, T.M., Schuitemaker, J.H., Kapsenberg, M.L., et al. (2007). Human keratinocytes express functional Toll-like receptor 3, 4, 5, and 9. *J Invest Dermatol* 127(2)**,** 331-341. doi: 10.1038/sj.jid.5700530.

Lee, J.K., Kim, S.H., Lewis, E.C., Azam, T., Reznikov, L.L., and Dinarello, C.A. (2004). Differences in signaling pathways by IL-1beta and IL-18. *Proc Natl Acad Sci U S A* 101(23)**,** 8815-8820. doi: 10.1073/pnas.0402800101.

Lee, K.G., Xu, S., Kang, Z.H., Huo, J., Huang, M., Liu, D., et al. (2012). Bruton's tyrosine kinase phosphorylates Toll-like receptor 3 to initiate antiviral response. *Proc Natl Acad Sci U S A* 109(15)**,** 5791-5796. doi: 10.1073/pnas.1119238109.

Lei, K., and Davis, R.J. (2003). JNK phosphorylation of Bim-related members of the Bcl2 family induces Bax-dependent apoptosis. *Proc Natl Acad Sci U S A* 100(5)**,** 2432-2437. doi: 10.1073/pnas.0438011100.

Lei, K., Nimnual, A., Zong, W.X., Kennedy, N.J., Flavell, R.A., Thompson, C.B., et al. (2002). The Bax subfamily of Bcl2-related proteins is essential for apoptotic signal transduction by c-Jun NH(2)-terminal kinase. *Mol Cell Biol* 22(13)**,** 4929-4942.

Leifer, C.A., and Medvedev, A.E. (2016). Molecular mechanisms of regulation of Toll-like receptor signaling. *J Leukoc Biol* 100(5)**,** 927-941. doi: 10.1189/jlb.2MR0316-117RR.

Li, Z., Jiang, Y., Ulevitch, R.J., and Han, J. (1996). The primary structure of p38 gamma: a new member of p38 group of MAP kinases. *Biochem Biophys Res Commun* 228(2)**,** 334-340. doi: 10.1006/bbrc.1996.1662.

Li, Z.J., Choi, D.K., Sohn, K.C., Seo, M.S., Lee, H.E., Lee, Y., et al. (2014). Propionibacterium acnes activates the NLRP3 inflammasome in human sebocytes. *J Invest Dermatol* 134(11)**,** 2747-2756. doi: 10.1038/jid.2014.221.

Lin, K.M., Hu, W., Troutman, T.D., Jennings, M., Brewer, T., Li, X., et al. (2014). IRAK-1 bypasses priming and directly links TLRs to rapid NLRP3 inflammasome activation. *Proc Natl Acad Sci U S A* 111(2)**,** 775-780. doi: 10.1073/pnas.1320294111.

Lin, Y.C., Huang, D.Y., Wang, J.S., Lin, Y.L., Hsieh, S.L., Huang, K.C., et al. (2015). Syk is involved in NLRP3 inflammasome-mediated caspase-1 activation through adaptor ASC phosphorylation and enhanced oligomerization. *J Leukoc Biol* 97(5)**,** 825-835. doi: 10.1189/jlb.3HI0814-371RR.

Lindor, N.M., Arsenault, T.M., Solomon, H., Seidman, C.E., and McEvoy, M.T. (1997). A new autosomal dominant disorder of pyogenic sterile arthritis, pyoderma gangrenosum, and acne: PAPA syndrome. *Mayo Clin Proc* 72(7)**,** 611-615. doi: 10.1016/S0025-6196(11)63565-9.

Liu, L., Rezvani, H.R., Back, J.H., Hosseini, M., Tang, X.W., Zhu, Y., et al. (2014). Inhibition of p38 MAPK Signaling Augments Skin Tumorigenesis via NOX2 Driven ROS Generation. *Plos One* 9(5). doi: ARTN e97245
10.1371/journal.pone.0097245.

Liu, R., Khalil, H., Lin, S.J., Sargent, M.A., York, A.J., and Molkentin, J.D. (2016). Nemo-Like Kinase (NLK) Is a Pathological Signaling Effector in the Mouse Heart. *PLoS One* 11(10)**,** e0164897. doi: 10.1371/journal.pone.0164897.

Lu, A., Li, Y., Schmidt, F.I., Yin, Q., Chen, S., Fu, T.M., et al. (2016). Molecular basis of caspase-1 polymerization and its inhibition by a new capping mechanism. *Nat Struct Mol Biol* 23(5)**,** 416-425. doi: 10.1038/nsmb.3199.

Lu, B., Nakamura, T., Inouye, K., Li, J., Tang, Y., Lundback, P., et al. (2012). Novel role of PKR in inflammasome activation and HMGB1 release. *Nature* 488(7413)**,** 670-674. doi: 10.1038/nature11290.

Lupfer, C., and Kanneganti, T.D. (2013). Unsolved Mysteries in NLR Biology. *Front Immunol* 4**,** 285. doi: 10.3389/fimmu.2013.00285.

MacLeod, A.S., Rudolph, R., Corriden, R., Ye, I., Garijo, O., and Havran, W.L. (2014). Skin-resident T cells sense ultraviolet radiation-induced injury and contribute to DNA repair. *J Immunol* 192(12)**,** 5695-5702. doi: 10.4049/jimmunol.1303297.

Maekawa, S., Ohto, U., Shibata, T., Miyake, K., and Shimizu, T. (2016). Crystal structure of NOD2 and its implications in human disease. *Nat Commun* 7**,** 11813. doi: 10.1038/ncomms11813.

Maksimovic, S., Nakatani, M., Baba, Y., Nelson, A.M., Marshall, K.L., Wellnitz, S.A., et al. (2014). Epidermal Merkel cells are mechanosensory cells that tune mammalian touch receptors. *Nature* 509(7502)**,** 617-621. doi: 10.1038/nature13250.

Mali, P., Esvelt, K.M., and Church, G.M. (2013). Cas9 as a versatile tool for engineering biology. *Nat Methods* 10(10)**,** 957-963. doi: 10.1038/nmeth.2649.

Malissen, B., Tamoutounour, S., and Henri, S. (2014). The origins and functions of dendritic cells and macrophages in the skin. *Nat Rev Immunol* 14(6)**,** 417-428. doi: 10.1038/nri3683.

Man, S.M., and Kanneganti, T.D. (2016). Converging roles of caspases in inflammasome activation, cell death and innate immunity. *Nat Rev Immunol* 16(1)**,** 7-21. doi: 10.1038/nri.2015.7.

Man, S.M., Tourlomousis, P., Hopkins, L., Monie, T.P., Fitzgerald, K.A., and Bryant, C.E. (2013). Salmonella infection induces recruitment of Caspase-8 to the inflammasome to modulate IL-1beta production. *J Immunol* 191(10)**,** 5239-5246. doi: 10.4049/jimmunol.1301581.

Mandey, S.H., Kuijk, L.M., Frenkel, J., and Waterham, H.R. (2006). A role for geranylgeranylation in interleukin-1beta secretion. *Arthritis Rheum* 54(11)**,** 3690-3695. doi: 10.1002/art.22194.

Manukyan, M., Triantafilou, K., Triantafilou, M., Mackie, A., Nilsen, N., Espevik, T., et al. (2005). Binding of lipopeptide to CD14 induces physical proximity of CD14, TLR2 and TLR1. *Eur J Immunol* 35(3)**,** 911-921. doi: 10.1002/eji.200425336.

Mao, K., Chen, S., Chen, M., Ma, Y., Wang, Y., Huang, B., et al. (2013). Nitric oxide suppresses NLRP3 inflammasome activation and protects against LPS-induced septic shock. *Cell Res* 23(2)**,** 201-212. doi: 10.1038/cr.2013.6.

Mariathasan, S., Newton, K., Monack, D.M., Vucic, D., French, D.M., Lee, W.P., et al. (2004). Differential activation of the inflammasome by caspase-1 adaptors ASC and Ipaf. *Nature* 430(6996)**,** 213-218. doi: 10.1038/nature02664.

Mariathasan, S., Weiss, D.S., Newton, K., McBride, J., O'Rourke, K., Roose-Girma, M., et al. (2006). Cryopyrin activates the inflammasome in response to toxins and ATP. *Nature* 440(7081)**,** 228-232. doi: 10.1038/nature04515.

Marino, D., Reichmann, E., and Meuli, M. (2014). Skingineering. *Eur J Pediatr Surg* 24(3)**,** 205-213. doi: 10.1055/s-0034-1376315.

Martin, B.N., Wang, C., Willette-Brown, J., Herjan, T., Gulen, M.F., Zhou, H., et al. (2014). IKKalpha negatively regulates ASC-dependent inflammasome activation. *Nat Commun* 5**,** 4977. doi: 10.1038/ncomms5977.

Martinon, F., Agostini, L., Meylan, E., and Tschopp, J. (2004). Identification of bacterial muramyl dipeptide as activator of the NALP3/cryopyrin inflammasome. *Curr Biol* 14(21)**,** 1929-1934. doi: 10.1016/j.cub.2004.10.027.

Martinon, F., Burns, K., and Tschopp, J. (2002). The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. *Mol Cell* 10(2)**,** 417-426.

Martinon, F., Petrilli, V., Mayor, A., Tardivel, A., and Tschopp, J. (2006). Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature* 440(7081)**,** 237-241. doi: 10.1038/nature04516.

Marx, V. (2014). Gene editing: how to stay on-target with CRISPR. *Nat Methods* 11(10)**,** 1021-1026. doi: 10.1038/nmeth.3108.

Marzano, A.V., Fanoni, D., Antiga, E., Quaglino, P., Caproni, M., Crosti, C., et al. (2014). Expression of cytokines, chemokines and other effector molecules in two prototypic autoinflammatory skin diseases, pyoderma gangrenosum and Sweet's syndrome. *Clin Exp Immunol* 178(1)**,** 48-56. doi: 10.1111/cei.12394.

Masters, S.L. (2013). Specific inflammasomes in complex diseases. *Clin Immunol* 147(3)**,** 223-228. doi: 10.1016/j.clim.2012.12.006.

Masters, S.L., Lagou, V., Jeru, I., Baker, P.J., Van Eyck, L., Parry, D.A., et al. (2016). Familial autoinflammation with neutrophilic dermatosis reveals a regulatory mechanism of pyrin activation. *Sci Transl Med* 8(332)**,** 332ra345. doi: 10.1126/scitranslmed.aaf1471.

Masumoto, J., Taniguchi, S., Ayukawa, K., Sarvotham, H., Kishino, T., Niikawa, N., et al. (1999). ASC, a novel 22-kDa protein, aggregates during apoptosis of human promyelocytic leukemia HL-60 cells. *J Biol Chem* 274(48)**,** 33835-33838.

Matsushima, H., Ogawa, Y., Miyazaki, T., Tanaka, H., Nishibu, A., and Takashima, A. (2010). Intravital imaging of IL-1beta production in skin. *J Invest Dermatol* 130(6)**,** 1571-1580. doi: 10.1038/jid.2010.11.

Matzinger, P. (1994). Tolerance, danger, and the extended family. *Annu Rev Immunol* 12**,** 991-1045. doi: 10.1146/annurev.iy.12.040194.005015.

Mavropoulos, A., Orfanidou, T., Liaskos, C., Smyk, D.S., Spyrou, V., Sakkas, L.I., et al. (2013). p38 MAPK Signaling in Pemphigus: Implications for Skin Autoimmunity. *Autoimmune Dis* 2013**,** 728529. doi: 10.1155/2013/728529.

McDermott, M.F., Aksentijevich, I., Galon, J., McDermott, E.M., Ogunkolade, B.W., Centola, M., et al. (1999). Germline mutations in the extracellular domains of the 55 kDa TNF receptor, TNFR1, define a family of dominantly inherited autoinflammatory syndromes. *Cell* 97(1)**,** 133-144.

McGrath, J.A., and Uitto, J. (2016). "Structure and Function of the Skin," in *Rook's Textbook of Dermatology, Ninth Edition*. John Wiley & Sons, Ltd).

McLean, W.H., and Moore, C.B. (2011). Keratin disorders: from gene to therapy. *Hum Mol Genet* 20(R2)**,** R189-197. doi: 10.1093/hmg/ddr379.

Meixenberger, K., Pache, F., Eitel, J., Schmeck, B., Hippenstiel, S., Slevogt, H., et al. (2010). Listeria monocytogenes-infected human peripheral blood mononuclear cells produce IL-1beta, depending on listeriolysin O and NLRP3. *J Immunol* 184(2)**,** 922-930. doi: 10.4049/jimmunol.0901346.

Menon, G.K., Ghadially, R., Williams, M.L., and Elias, P.M. (1992). Lamellar bodies as delivery systems of hydrolytic enzymes: implications for normal and abnormal desquamation. *Br J Dermatol* 126(4)**,** 337-345.

Merad, M., Ginhoux, F., and Collin, M. (2008). Origin, homeostasis and function of Langerhans cells and other langerin-expressing dendritic cells. *Nat Rev Immunol* 8(12)**,** 935-947. doi: 10.1038/nri2455.

Miao, E.A., Mao, D.P., Yudkovsky, N., Bonneau, R., Lorang, C.G., Warren, S.E., et al. (2010). Innate immune detection of the type III secretion apparatus through the NLRC4 inflammasome. *Proc Natl Acad Sci U S A* 107(7)**,** 3076-3080. doi: 10.1073/pnas.0913087107.

Miceli-Richard, C., Lesage, S., Rybojad, M., Prieur, A.M., Manouvrier-Hanu, S., Hafner, R., et al. (2001). CARD15 mutations in Blau syndrome. *Nat Genet* 29(1)**,** 19-20. doi: 10.1038/ng720.

Mihara, K., Elliott, G.R., Boots, A.M., and Nelissen, R.L. (2012). Inhibition of p38 kinase suppresses the development of psoriasis-like lesions in a human skin transplant model of psoriasis. *Br J Dermatol* 167(2)**,** 455-457. doi: 10.1111/j.1365-2133.2012.10939.x.

Mishra, B.B., Rathinam, V.A., Martens, G.W., Martinot, A.J., Kornfeld, H., Fitzgerald, K.A., et al. (2013). Nitric oxide controls the immunopathology of tuberculosis by inhibiting NLRP3 inflammasome-dependent processing of IL-1beta. *Nat Immunol* 14(1)**,** 52-60. doi: 10.1038/ni.2474.

Moiani, A., Paleari, Y., Sartori, D., Mezzadra, R., Miccio, A., Cattoglio, C., et al. (2012). Lentiviral vector integration in the human genome induces alternative splicing and generates aberrant transcripts. *J Clin Invest* 122(5)**,** 1653-1666. doi: 10.1172/JCI61852.

Moro, K., and Koyasu, S. (2015). Innate lymphoid cells, possible interaction with microbiota. *Semin Immunopathol* 37(1)**,** 27-37. doi: 10.1007/s00281-014-0470-4.

Mose, M., Kang, Z., Raaby, L., Iversen, L., and Johansen, C. (2013). TNFalpha- and IL-17A-mediated S100A8 expression is regulated by p38 MAPK. *Exp Dermatol* 22(7)**,** 476-481. doi: 10.1111/exd.12187.

Mueller, S.N., Zaid, A., and Carbone, F.R. (2014). Tissue-resident T cells: dynamic players in skin immunity. *Front Immunol* 5**,** 332. doi: 10.3389/fimmu.2014.00332.

Munding, C., Keller, M., Niklaus, G., Papin, S., Tschopp, J., Werner, S., et al. (2006). The estrogen-responsive B box protein: a novel enhancer of interleukin-1beta secretion. *Cell Death Differ* 13(11)**,** 1938-1949. doi: 10.1038/sj.cdd.4401896.

Munoz-Planillo, R., Franchi, L., Miller, L.S., and Nunez, G. (2009). A critical role for hemolysins and bacterial lipoproteins in Staphylococcus aureus-induced activation of the Nlrp3 inflammasome. *J Immunol* 183(6)**,** 3942-3948. doi: 10.4049/jimmunol.0900729.

Munoz-Planillo, R., Kuffa, P., Martinez-Colon, G., Smith, B.L., Rajendiran, T.M., and Nunez, G. (2013). K(+) efflux is the common trigger of NLRP3 inflammasome activation by bacterial toxins and particulate matter. *Immunity* 38(6)**,** 1142-1153. doi: 10.1016/j.immuni.2013.05.016.

Murakami, T., Ockinger, J., Yu, J., Byles, V., McColl, A., Hofer, A.M., et al. (2012). Critical role for calcium mobilization in activation of the NLRP3 inflammasome. *Proc Natl Acad Sci U S A* 109(28)**,** 11282-11287. doi: 10.1073/pnas.1117765109.

Murphy, K., and Weaver, C. (2016). *Janeway's immunobiology*. New York, NY: Garland Science/Taylor & Francis Group, LLC.

Muruve, D.A., Petrilli, V., Zaiss, A.K., White, L.R., Clark, S.A., Ross, P.J., et al. (2008). The inflammasome recognizes cytosolic microbial and host DNA and triggers an innate immune response. *Nature* 452(7183)**,** 103-107. doi: 10.1038/nature06664.

Muthusamy, V., and Piva, T.J. (2013). A comparative study of UV-induced cell signalling pathways in human keratinocyte-derived cell lines. *Arch Dermatol Res* 305(9)**,** 817-833. doi: 10.1007/s00403-013-1412-z.

Nakamura, K., Okamura, H., Nagata, K., Komatsu, T., and Tamura, T. (1993). Purification of a factor which provides a costimulatory signal for gamma interferon production. *Infect Immun* 61(1)**,** 64-70.

Nakamura, K., Okamura, H., Wada, M., Nagata, K., and Tamura, T. (1989). Endotoxin-induced serum factor that stimulates gamma interferon production. *Infect Immun* 57(2)**,** 590-595.

Nakamura, S., Takahashi, H., Kinouchi, M., Manabe, A., Ishida-Yamamoto, A., Hashimoto, Y., et al. (2001). Differential phosphorylation of mitogen-activated protein kinase families by epidermal growth factor and ultraviolet B irradiation in SV40-transformed human keratinocytes. *J Dermatol Sci* 25(2)**,** 139-149.

Navarini, A.A., Satoh, T.K., and French, L.E. (2016). Neutrophilic dermatoses and autoinflammatory diseases with skin involvement--innate immune disorders. *Semin Immunopathol* 38(1)**,** 45-56. doi: 10.1007/s00281-015-0549-6.

Nestle, F.O., Di Meglio, P., Qin, J.Z., and Nickoloff, B.J. (2009). Skin immune sentinels in health and disease. *Nat Rev Immunol* 9(10)**,** 679-691. doi: 10.1038/nri2622.

Netea, M.G., van de Veerdonk, F.L., van der Meer, J.W., Dinarello, C.A., and Joosten, L.A. (2015). Inflammasome-independent regulation of IL-1-family cytokines. *Annu Rev Immunol* 33**,** 49-77. doi: 10.1146/annurev-immunol-032414-112306.

Neumann, K., and Ruland, J. (2013). Kinases conquer the inflammasomes. *Nat Immunol* 14(12)**,** 1207-1208. doi: 10.1038/ni.2763.

Niessen, C.M. (2007). Tight junctions/adherens junctions: basic structure and function. *J Invest Dermatol* 127(11)**,** 2525-2532. doi: 10.1038/sj.jid.5700865.

Noel, F., Pierard-Franchimont, C., Pierard, G.E., and Quatresooz, P. (2012). Sweaty skin, background and assessments. *Int J Dermatol* 51(6)**,** 647-655. doi: 10.1111/j.1365-4632.2011.05307.x.

Nyman, T.A., Lorey, M.B., Cypryk, W., and Matikainen, S. (2017). Mass spectrometry-based proteomic exploration of the human immune system: focus on the inflammasome, global protein secretion, and T cells. *Expert Rev Proteomics* 14(5)**,** 395-407. doi: 10.1080/14789450.2017.1319768.

Oji, V., Metze, D., and Traupe, H. (2016). "Inherited Disorders of Cornification," in *Rook's Textbook of Dermatology*. John Wiley & Sons, Ltd).

Okada, M., Matsuzawa, A., Yoshimura, A., and Ichijo, H. (2014). The lysosome rupture-activated TAK1-JNK pathway regulates NLRP3 inflammasome activation. *J Biol Chem* 289(47)**,** 32926-32936. doi: 10.1074/jbc.M114.579961.

Oosting, M., Cheng, S.C., Bolscher, J.M., Vestering-Stenger, R., Plantinga, T.S., Verschueren, I.C., et al. (2014). Human TLR10 is an anti-inflammatory pattern-recognition receptor. *Proc Natl Acad Sci U S A* 111(42)**,** E4478-4484. doi: 10.1073/pnas.1410293111.

Pan, X., Hobbs, R.P., and Coulombe, P.A. (2013). The expanding significance of keratin intermediate filaments in normal and diseased epithelia. *Curr Opin Cell Biol* 25(1)**,** 47-56. doi: 10.1016/j.ceb.2012.10.018.

Park, H., Iqbal, S., Hernandez, P., Mora, R., Zheng, K., Feng, Y., et al. (2015). Structural basis and biological consequences for JNK2/3 isoform selective aminopyrazoles. *Sci Rep* 5**,** 8047. doi: 10.1038/srep08047.

Park, J.M., Greten, F.R., Li, Z.W., and Karin, M. (2002). Macrophage apoptosis by anthrax lethal factor through p38 MAP kinase inhibition. *Science* 297(5589)**,** 2048-2051. doi: 10.1126/science.1073163.

Park, Y.H., Wood, G., Kastner, D.L., and Chae, J.J. (2016). Pyrin inflammasome activation and RhoA signaling in the autoinflammatory diseases FMF and HIDS. *Nat Immunol* 17(8)**,** 914-921. doi: 10.1038/ni.3457.

Pasparakis, M., Haase, I., and Nestle, F.O. (2014). Mechanisms regulating skin immunity and inflammation. *Nat Rev Immunol* 14(5)**,** 289-301. doi: 10.1038/nri3646.

Perera, G.K., Di Meglio, P., and Nestle, F.O. (2012). Psoriasis. *Annu Rev Pathol* 7**,** 385-422. doi: 10.1146/annurev-pathol-011811-132448.

Petrilli, V., Papin, S., Dostert, C., Mayor, A., Martinon, F., and Tschopp, J. (2007). Activation of the NALP3 inflammasome is triggered by low intracellular potassium concentration. *Cell Death Differ* 14(9)**,** 1583-1589. doi: 10.1038/sj.cdd.4402195.

Pfundt, R., van Vlijmen-Willems, I., Bergers, M., Wingens, M., Cloin, W., and Schalkwijk, J. (2001). In situ demonstration of phosphorylated c-jun and p38 MAP kinase in epidermal keratinocytes following ultraviolet B irradiation of human skin. *J Pathol* 193(2)**,** 248-255. doi: 10.1002/1096-9896(2000)9999:9999<::AID-PATH780>3.0.CO;2-Y.

Philpott, D.J., Sorbara, M.T., Robertson, S.J., Croitoru, K., and Girardin, S.E. (2014). NOD proteins: regulators of inflammation in health and disease. *Nat Rev Immunol* 14(1)**,** 9-23. doi: 10.1038/nri3565.

Pilla, D.M., Hagar, J.A., Haldar, A.K., Mason, A.K., Degrandi, D., Pfeffer, K., et al. (2014). Guanylate binding proteins promote caspase-11-dependent pyroptosis in response to cytoplasmic LPS. *Proc Natl Acad Sci U S A* 111(16)**,** 6046-6051. doi: 10.1073/pnas.1321700111.

Porter, K.A., Duffy, E.B., Nyland, P., Atianand, M.K., Sharifi, H., and Harton, J.A. (2014). The CLRX.1/NOD24 (NLRP2P) pseudogene codes a functional negative regulator of NF-kappaB, pyrin-only protein 4. *Genes Immun* 15(6)**,** 392-403. doi: 10.1038/gene.2014.30.

Poumay, Y., and Pittelkow, M.R. (1995). Cell density and culture factors regulate keratinocyte commitment to differentiation and expression of suprabasal K1/K10 keratins. *J Invest Dermatol* 104(2)**,** 271-276.

Py, B.F., Kim, M.S., Vakifahmetoglu-Norberg, H., and Yuan, J. (2013). Deubiquitination of NLRP3 by BRCC3 critically regulates inflammasome activity. *Mol Cell* 49(2)**,** 331-338. doi: 10.1016/j.molcel.2012.11.009.

Qin, H., Jin, J., Fischer, H., Mildner, M., Gschwandtner, M., Mlitz, V., et al. (2017). The caspase-1 inhibitor CARD18 is specifically expressed during late differentiation of keratinocytes and its expression is lost in lichen planus. *J Dermatol Sci*. doi: 10.1016/j.jdermsci.2017.04.015.

Qin, M., Pirouz, A., Kim, M.H., Krutzik, S.R., Garban, H.J., and Kim, J. (2014). Propionibacterium acnes Induces IL-1beta secretion via the NLRP3 inflammasome in human monocytes. *J Invest Dermatol* 134(2)**,** 381-388. doi: 10.1038/jid.2013.309.

Qu, Y., Misaghi, S., Izrael-Tomasevic, A., Newton, K., Gilmour, L.L., Lamkanfi, M., et al. (2012). Phosphorylation of NLRC4 is critical for inflammasome activation. *Nature* 490(7421)**,** 539-542. doi: 10.1038/nature11429.

Qu, Y., Misaghi, S., Newton, K., Maltzman, A., Izrael-Tomasevic, A., Arnott, D., et al. (2016). NLRP3 recruitment by NLRC4 during Salmonella infection. *J Exp Med* 213(6)**,** 877-885. doi: 10.1084/jem.20132234.

Rabeony, H., Pohin, M., Vasseur, P., Petit-Paris, I., Jegou, J.F., Favot, L., et al. (2015). IMQ-induced skin inflammation in mice is dependent on IL-1R1 and MyD88 signaling but independent of the NLRP3 inflammasome. *Eur J Immunol* 45(10)**,** 2847-2857. doi: 10.1002/eji.201445215.

Raingeaud, J., Whitmarsh, A.J., Barrett, T., Derijard, B., and Davis, R.J. (1996). MKK3- and MKK6-regulated gene expression is mediated by the p38 mitogen-activated protein kinase signal transduction pathway. *Mol Cell Biol* 16(3)**,** 1247-1255.

Ran, F.A., Hsu, P.D., Wright, J., Agarwala, V., Scott, D.A., and Zhang, F. (2013). Genome engineering using the CRISPR-Cas9 system. *Nat Protoc* 8(11)**,** 2281-2308. doi: 10.1038/nprot.2013.143.

Rasmussen, C., Thomas-Virnig, C., and Allen-Hoffmann, B.L. (2013). Classical human epidermal keratinocyte cell culture. *Methods Mol Biol* 945**,** 161-175. doi: 10.1007/978-1-62703-125-7_11.

Ratsimandresey, R.A., Chu, L.H., Khare, S., de Almeida, L., Gangopadhyay, A., Indramohan, M., et al. (2017). The PYRIN domain-only protein POP2 inhibits inflammasome priming and activation. *Nat Commun* 8**,** 15556. doi: 10.1038/ncomms15556.

Reddy, S., Jia, S., Geoffrey, R., Lorier, R., Suchi, M., Broeckel, U., et al. (2009). An autoinflammatory disease due to homozygous deletion of the IL1RN locus. *N Engl J Med* 360(23)**,** 2438-2444. doi: 10.1056/NEJMoa0809568.

Reikine, S., Nguyen, J.B., and Modis, Y. (2014). Pattern Recognition and Signaling Mechanisms of RIG-I and MDA5. *Front Immunol* 5**,** 342. doi: 10.3389/fimmu.2014.00342.

Reinholz, M., Kawakami, Y., Salzer, S., Kreuter, A., Dombrowski, Y., Koglin, S., et al. (2013). HPV16 activates the AIM2 inflammasome in keratinocytes. *Arch Dermatol Res* 305(8)**,** 723-732. doi: 10.1007/s00403-013-1375-0.

Rheinwald, J.G. (1980). Serial cultivation of normal human epidermal keratinocytes. *Methods Cell Biol* 21A**,** 229-254.

Rheinwald, J.G., and Green, H. (1975). Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells. *Cell* 6(3)**,** 331-343.

Romberg, N., Al Moussawi, K., Nelson-Williams, C., Stiegler, A.L., Loring, E., Choi, M., et al. (2014). Mutation of NLRC4 causes a syndrome of enterocolitis and autoinflammation. *Nat Genet* 46(10)**,** 1135-1139. doi: 10.1038/ng.3066.

Rosenwasser, L.J., Dinarello, C.A., and Rosenthal, A.S. (1979). Adherent cell function in murine T-lymphocyte antigen recognition. IV. Enhancement of murine T-cell antigen recognition by human leukocytic pyrogen. *J Exp Med* 150(3)**,** 709-714.

Rosette, C., and Karin, M. (1996). Ultraviolet light and osmotic stress: activation of the JNK cascade through multiple growth factor and cytokine receptors. *Science* 274(5290)**,** 1194-1197.

Rothnagel, J.A., Dominey, A.M., Dempsey, L.D., Longley, M.A., Greenhalgh, D.A., Gagne, T.A., et al. (1992). Mutations in the rod domains of keratins 1 and 10 in epidermolytic hyperkeratosis. *Science* 257(5073)**,** 1128-1130.

Saba-El-Leil, M.K., Fremin, C., and Meloche, S. (2016). Redundancy in the World of MAP Kinases: All for One. *Front Cell Dev Biol* 4**,** 67. doi: 10.3389/fcell.2016.00067.

Sabbah, A., Chang, T.H., Harnack, R., Frohlich, V., Tominaga, K., Dube, P.H., et al. (2009). Activation of innate immune antiviral responses by Nod2. *Nat Immunol* 10(10)**,** 1073-1080. doi: 10.1038/ni.1782.

Sahillioglu, A.C., Sumbul, F., Ozoren, N., and Haliloglu, T. (2014). Structural and dynamics aspects of ASC speck assembly. *Structure* 22(12)**,** 1722-1734. doi: 10.1016/j.str.2014.09.011.

Saito, T., Owen, D.M., Jiang, F., Marcotrigiano, J., and Gale, M., Jr. (2008). Innate immunity induced by composition-dependent RIG-I recognition of hepatitis C virus RNA. *Nature* 454(7203)**,** 523-527. doi: 10.1038/nature07106.

Samali, A., Zhivotovsky, B., Jones, D., Nagata, S., and Orrenius, S. (1999). Apoptosis: cell death defined by caspase activation. *Cell Death Differ* 6(6)**,** 495-496. doi: 10.1038/sj.cdd.4400520.

Sanjana, N.E., Shalem, O., and Zhang, F. (2014). Improved vectors and genome-wide libraries for CRISPR screening. *Nat Methods* 11(8)**,** 783-784. doi: 10.1038/nmeth.3047.

Satoh, T., Kambe, N., and Matsue, H. (2013). NLRP3 activation induces ASC-dependent programmed necrotic cell death, which leads to neutrophilic inflammation. *Cell Death Dis* 4**,** e644. doi: 10.1038/cddis.2013.169.

Satoh, T., Otsuka, A., Contassot, E., and French, L.E. (2015). The inflammasome and IL-1beta: implications for the treatment of inflammatory diseases. *Immunotherapy* 7(3)**,** 243-254. doi: 10.2217/imt.14.106.

Savina, A., and Amigorena, S. (2007). Phagocytosis and antigen presentation in dendritic cells. *Immunol Rev* 219**,** 143-156. doi: 10.1111/j.1600-065X.2007.00552.x.

Schmid-Burgk, J.L., Chauhan, D., Schmidt, T., Ebert, T.S., Reinhardt, J., Endl, E., et al. (2016). A Genome-wide CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) Screen Identifies NEK7 as an Essential Component of NLRP3 Inflammasome Activation. *J Biol Chem* 291(1)**,** 103-109. doi: 10.1074/jbc.C115.700492.

Schneider, M., Zimmermann, A.G., Roberts, R.A., Zhang, L., Swanson, K.V., Wen, H., et al. (2012). The innate immune sensor NLRC3 attenuates Toll-like receptor signaling via modification of the signaling adaptor TRAF6 and transcription factor NF-kappaB. *Nat Immunol* 13(9)**,** 823-831. doi: 10.1038/ni.2378.

Schreiber, S., Kilgus, O., Payer, E., Kutil, R., Elbe, A., Mueller, C., et al. (1992). Cytokine pattern of Langerhans cells isolated from murine epidermal cell cultures. *J Immunol* 149(11)**,** 3524-3534.

Schroder, K., and Tschopp, J. (2010). The inflammasomes. *Cell* 140(6)**,** 821-832. doi: 10.1016/j.cell.2010.01.040.

Seger, R., and Krebs, E.G. (1995). The MAPK signaling cascade. *FASEB J* 9(9)**,** 726-735.

Serruto, D., Rappuoli, R., Scarselli, M., Gros, P., and van Strijp, J.A. (2010). Molecular mechanisms of complement evasion: learning from staphylococci and meningococci. *Nat Rev Microbiol* 8(6)**,** 393-399. doi: 10.1038/nrmicro2366.

Sester, D.P., Thygesen, S.J., Sagulenko, V., Vajjhala, P.R., Cridland, J.A., Vitak, N., et al. (2015). A novel flow cytometric method to assess inflammasome formation. *J Immunol* 194(1)**,** 455-462. doi: 10.4049/jimmunol.1401110.

Shen, B., Zhang, J., Wu, H., Wang, J., Ma, K., Li, Z., et al. (2013). Generation of gene-modified mice via Cas9/RNA-mediated gene targeting. *Cell Res* 23(5)**,** 720-723. doi: 10.1038/cr.2013.46.

Shi, H., Wang, Y., Li, X., Zhan, X., Tang, M., Fina, M., et al. (2016). NLRP3 activation and mitosis are mutually exclusive events coordinated by NEK7, a new inflammasome component. *Nat Immunol* 17(3)**,** 250-258. doi: 10.1038/ni.3333.

Shi, J., Zhao, Y., Wang, K., Shi, X., Wang, Y., Huang, H., et al. (2015). Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. *Nature* 526(7575)**,** 660-665. doi: 10.1038/nature15514.

Shi, J., Zhao, Y., Wang, Y., Gao, W., Ding, J., Li, P., et al. (2014). Inflammatory caspases are innate immune receptors for intracellular LPS. *Nature* 514(7521)**,** 187-192. doi: 10.1038/nature13683.

Shimada, K., Crother, T.R., Karlin, J., Dagvadorj, J., Chiba, N., Chen, S., et al. (2012). Oxidized mitochondrial DNA activates the NLRP3 inflammasome during apoptosis. *Immunity* 36(3)**,** 401-414. doi: 10.1016/j.immuni.2012.01.009.

Shinkuma, S., Guo, Z., and Christiano, A.M. (2016). Site-specific genome editing for correction of induced pluripotent stem cells derived from dominant dystrophic epidermolysis bullosa. *Proc Natl Acad Sci U S A* 113(20)**,** 5676-5681. doi: 10.1073/pnas.1512028113.

Shio, M.T., Christian, J.G., Jung, J.Y., Chang, K.P., and Olivier, M. (2015). PKC/ROS-Mediated NLRP3 Inflammasome Activation Is Attenuated by Leishmania Zinc-Metalloprotease during Infection. *PLoS Negl Trop Dis* 9(6)**,** e0003868. doi: 10.1371/journal.pntd.0003868.

Shio, M.T., Eisenbarth, S.C., Savaria, M., Vinet, A.F., Bellemare, M.J., Harder, K.W., et al. (2009). Malarial hemozoin activates the NLRP3 inflammasome through Lyn and Syk kinases. *PLoS Pathog* 5(8)**,** e1000559. doi: 10.1371/journal.ppat.1000559.

Shoham, N.G., Centola, M., Mansfield, E., Hull, K.M., Wood, G., Wise, C.A., et al. (2003). Pyrin binds the PSTPIP1/CD2BP1 protein, defining familial Mediterranean fever and PAPA syndrome as disorders in the same pathway. *Proc Natl Acad Sci U S A* 100(23)**,** 13501-13506. doi: 10.1073/pnas.2135380100.

Shornick, L.P., Bisarya, A.K., and Chaplin, D.D. (2001). IL-1beta is essential for langerhans cell activation and antigen delivery to the lymph nodes during contact sensitization: evidence for a dermal source of IL-1beta. *Cell Immunol* 211(2)**,** 105-112. doi: 10.1006/cimm.2001.1834.

Sollberger, G., Strittmatter, G.E., Grossi, S., Garstkiewicz, M., Auf dem Keller, U., French, L.E., et al. (2015). Caspase-1 activity is required for UVB-induced apoptosis of human keratinocytes. *J Invest Dermatol* 135(5)**,** 1395-1404. doi: 10.1038/jid.2014.551.

Sollberger, G., Strittmatter, G.E., Kistowska, M., French, L.E., and Beer, H.D. (2012). Caspase-4 is required for activation of inflammasomes. *J Immunol* 188(4)**,** 1992-2000. doi: 10.4049/jimmunol.1101620.

Soong, G., Chun, J., Parker, D., and Prince, A. (2012). Staphylococcus aureus activation of caspase 1/calpain signaling mediates invasion through human keratinocytes. *J Infect Dis* 205(10)**,** 1571-1579. doi: 10.1093/infdis/jis244.

Spalinger, M.R., Kasper, S., Gottier, C., Lang, S., Atrott, K., Vavricka, S.R., et al. (2016). NLRP3 tyrosine phosphorylation is controlled by protein tyrosine phosphatase PTPN22. *J Clin Invest* 126(5)**,** 1783-1800. doi: 10.1172/JCI83669.

Stoitzner, P., Tripp, C.H., Douillard, P., Saeland, S., and Romani, N. (2005). Migratory Langerhans cells in mouse lymph nodes in steady state and inflammation. *J Invest Dermatol* 125(1)**,** 116-125. doi: 10.1111/j.0022-202X.2005.23757.x.

Stoitzner, P., Tripp, C.H., Eberhart, A., Price, K.M., Jung, J.Y., Bursch, L., et al. (2006). Langerhans cells cross-present antigen derived from skin. *Proc Natl Acad Sci U S A* 103(20)**,** 7783-7788. doi: 10.1073/pnas.0509307103.

Stone, K.D., Prussin, C., and Metcalfe, D.D. (2010). IgE, mast cells, basophils, and eosinophils. *J Allergy Clin Immunol* 125(2 Suppl 2)**,** S73-80. doi: 10.1016/j.jaci.2009.11.017.

Strasser, D., Neumann, K., Bergmann, H., Marakalala, M.J., Guler, R., Rojowska, A., et al. (2012). Syk kinase-coupled C-type lectin receptors engage protein kinase C-sigma to elicit Card9 adaptor-mediated innate immunity. *Immunity* 36(1)**,** 32-42. doi: 10.1016/j.immuni.2011.11.015.

Strittmatter, G.E., Sand, J., Sauter, M., Seyffert, M., Steigerwald, R., Fraefel, C., et al. (2016). IFN-gamma Primes Keratinocytes for HSV-1-Induced Inflammasome Activation. *J Invest Dermatol* 136(3)**,** 610-620. doi: 10.1016/j.jid.2015.12.022.

Stutz, A., Horvath, G.L., Monks, B.G., and Latz, E. (2013). ASC speck formation as a readout for inflammasome activation. *Methods Mol Biol* 1040**,** 91-101. doi: 10.1007/978-1-62703-523-1_8.

Stutz, A., Kolbe, C.C., Stahl, R., Horvath, G.L., Franklin, B.S., van Ray, O., et al. (2017). NLRP3 inflammasome assembly is regulated by phosphorylation of the pyrin domain. *J Exp Med* 214(6)**,** 1725-1736. doi: 10.1084/jem.20160933.

Sudo, T., Yagasaki, Y., Hama, H., Watanabe, N., and Osada, H. (2002). Exip, a new alternative splicing variant of p38 alpha, can induce an earlier onset of apoptosis in HeLa cells. *Biochem Biophys Res Commun* 291(4)**,** 838-843. doi: 10.1006/bbrc.2002.6529.

Sung, Y.H., Baek, I.J., Kim, D.H., Jeon, J., Lee, J., Lee, K., et al. (2013). Knockout mice created by TALEN-mediated gene targeting. *Nat Biotechnol* 31(1)**,** 23-24. doi: 10.1038/nbt.2477.

Symons, J.A., Young, P.R., and Duff, G.W. (1995). Soluble type II interleukin 1 (IL-1) receptor binds and blocks processing of IL-1 beta precursor and loses affinity for IL-1 receptor antagonist. *Proc Natl Acad Sci U S A* 92(5)**,** 1714-1718.

Takagi, A., Nishiyama, C., Kanada, S., Niwa, Y., Fukuyama, K., Ikeda, S., et al. (2006). Prolonged MHC class II expression and CIITA transcription in human keratinocytes. *Biochem Biophys Res Commun* 347(2)**,** 388-393. doi: 10.1016/j.bbrc.2006.05.215.

Takei, S., Hoshino, T., Matsunaga, K., Sakazaki, Y., Sawada, M., Oda, H., et al. (2011). Soluble interleukin-18 receptor complex is a novel biomarker in rheumatoid arthritis. *Arthritis Res Ther* 13(2)**,** R52. doi: 10.1186/ar3295.

Tan, Y., Rouse, J., Zhang, A., Cariati, S., Cohen, P., and Comb, M.J. (1996). FGF and stress regulate CREB and ATF-1 via a pathway involving p38 MAP kinase and MAPKAP kinase-2. *EMBO J* 15(17)**,** 4629-4642.

Tanoue, T., and Nishida, E. (2003). Molecular recognitions in the MAP kinase cascades. *Cell Signal* 15(5)**,** 455-462.

Terns, M.P., and Terns, R.M. (2011). CRISPR-based adaptive immune systems. *Curr Opin Microbiol* 14(3)**,** 321-327. doi: 10.1016/j.mib.2011.03.005.

Tominaga, K., Yoshimoto, T., Torigoe, K., Kurimoto, M., Matsui, K., Hada, T., et al. (2000). IL-12 synergizes with IL-18 or IL-1beta for IFN-gamma production from human T cells. *Int Immunol* 12(2)**,** 151-160.

Travassos, L.H., Carneiro, L.A., Ramjeet, M., Hussey, S., Kim, Y.G., Magalhaes, J.G., et al. (2010). Nod1 and Nod2 direct autophagy by recruiting ATG16L1 to the plasma membrane at the site of bacterial entry. *Nat Immunol* 11(1)**,** 55-62. doi: 10.1038/ni.1823.

Truett, G.E., Heeger, P., Mynatt, R.L., Truett, A.A., Walker, J.A., and Warman, M.L. (2000). Preparation of PCR-quality mouse genomic DNA with hot sodium hydroxide and tris (HotSHOT). *Biotechniques* 29(1)**,** 52, 54.

Tullett, K.M., Lahoud, M.H., and Radford, K.J. (2014). Harnessing Human Cross-Presenting CLEC9A(+)XCR1(+) Dendritic Cells for Immunotherapy. *Front Immunol* 5**,** 239. doi: 10.3389/fimmu.2014.00239.

Tzeng, T.C., Schattgen, S., Monks, B., Wang, D., Cerny, A., Latz, E., et al. (2016). A Fluorescent Reporter Mouse for Inflammasome Assembly Demonstrates an Important Role for Cell-Bound and Free ASC Specks during In Vivo Infection. *Cell Rep* 16(2)**,** 571-582. doi: 10.1016/j.celrep.2016.06.011.

Unterholzner, L., Keating, S.E., Baran, M., Horan, K.A., Jensen, S.B., Sharma, S., et al. (2010). IFI16 is an innate immune sensor for intracellular DNA. *Nat Immunol* 11(11)**,** 997-1004. doi: 10.1038/ni.1932.

Ushio, S., Namba, M., Okura, T., Hattori, K., Nukada, Y., Akita, K., et al. (1996). Cloning of the cDNA for human IFN-gamma-inducing factor, expression in Escherichia coli, and studies on the biologic activities of the protein. *J Immunol* 156(11)**,** 4274-4279.

Valerie, K., and Povirk, L.F. (2003). Regulation and mechanisms of mammalian double-strand break repair. *Oncogene* 22(37)**,** 5792-5812. doi: 10.1038/sj.onc.1206679.

van der Burgh, R., Ter Haar, N.M., Boes, M.L., and Frenkel, J. (2013). Mevalonate kinase deficiency, a metabolic autoinflammatory disease. *Clin Immunol* 147(3)**,** 197-206. doi: 10.1016/j.clim.2012.09.011.

van der Meer, J.W., Vossen, J.M., Radl, J., van Nieuwkoop, J.A., Meyer, C.J., Lobatto, S., et al. (1984). Hyperimmunoglobulinaemia D and periodic fever: a new syndrome. *Lancet* 1(8386)**,** 1087-1090.

Vladimer, G.I., Weng, D., Paquette, S.W., Vanaja, S.K., Rathinam, V.A., Aune, M.H., et al. (2012). The NLRP12 inflammasome recognizes Yersinia pestis. *Immunity* 37(1)**,** 96-107. doi: 10.1016/j.immuni.2012.07.006.

Voskoboinik, I., Whisstock, J.C., and Trapani, J.A. (2015). Perforin and granzymes: function, dysfunction and human pathology. *Nat Rev Immunol* 15(6)**,** 388-400. doi: 10.1038/nri3839.

Walko, G., Castanon, M.J., and Wiche, G. (2015). Molecular architecture and function of the hemidesmosome. *Cell Tissue Res* 360(3)**,** 529-544. doi: 10.1007/s00441-015-2216-6.

Walsh, J.G., Muruve, D.A., and Power, C. (2014). Inflammasomes in the CNS. *Nat Rev Neurosci* 15(2)**,** 84-97. doi: 10.1038/nrn3638.

Wang, C., Deng, L., Hong, M., Akkaraju, G.R., Inoue, J., and Chen, Z.J. (2001). TAK1 is a ubiquitin-dependent kinase of MKK and IKK. *Nature* 412(6844)**,** 346-351. doi: 10.1038/35085597.

Wang, H., La Russa, M., and Qi, L.S. (2016a). CRISPR/Cas9 in Genome Editing and Beyond. *Annu Rev Biochem* 85**,** 227-264. doi: 10.1146/annurev-biochem-060815-014607.

Wang, J.J., Tasinato, A., Ethell, D.W., Testa, M.P., and Bredesen, D.E. (2000). Phosphorylation of the common neurotrophin receptor p75 by p38beta2 kinase affects NF-kappaB and AP-1 activities. *J Mol Neurosci* 15(1)**,** 19-29. doi: 10.1385/JMN:15:1:19.

Wang, Y., Bugatti, M., Ulland, T.K., Vermi, W., Gilfillan, S., and Colonna, M. (2016b). Nonredundant roles of keratinocyte-derived IL-34 and neutrophil-derived CSF1 in Langerhans cell renewal in the steady state and during inflammation. *Eur J Immunol* 46(3)**,** 552-559. doi: 10.1002/eji.201545917.

Waskiewicz, A.J., Flynn, A., Proud, C.G., and Cooper, J.A. (1997). Mitogen-activated protein kinases activate the serine/threonine kinases Mnk1 and Mnk2. *EMBO J* 16(8)**,** 1909-1920. doi: 10.1093/emboj/16.8.1909.

Watanabe, H., Gaide, O., Petrilli, V., Martinon, F., Contassot, E., Roques, S., et al. (2007). Activation of the IL-1beta-processing inflammasome is involved in contact hypersensitivity. *J Invest Dermatol* 127(8)**,** 1956-1963. doi: 10.1038/sj.jid.5700819.

Webber, B.R., Osborn, M.J., McElroy, A.N., Twaroski, K., Lonetree, C.L., DeFeo, A.P., et al. (2016). CRISPR/Cas9-based genetic correction for recessive dystrophic epidermolysis bullosa. *NPJ Regen Med* 1. doi: 10.1038/npjregenmed.2016.14.

Weber, A., Wasiliew, P., and Kracht, M. (2010). Interleukin-1 (IL-1) pathway. *Sci Signal* 3(105)**,** cm1. doi: 10.1126/scisignal.3105cm1.

Werman, A., Werman-Venkert, R., White, R., Lee, J.K., Werman, B., Krelin, Y., et al. (2004). The precursor form of IL-1alpha is an intracrine proinflammatory activator of transcription. *Proc Natl Acad Sci U S A* 101(8)**,** 2434-2439.

Wiggin, G.R., Soloaga, A., Foster, J.M., Murray-Tait, V., Cohen, P., and Arthur, J.S. (2002). MSK1 and MSK2 are required for the mitogen- and stress-induced phosphorylation of CREB and ATF1 in fibroblasts. *Mol Cell Biol* 22(8)**,** 2871-2881.

Williams, K.L., Lich, J.D., Duncan, J.A., Reed, W., Rallabhandi, P., Moore, C., et al. (2005). The CATERPILLER protein monarch-1 is an antagonist of toll-like receptor-, tumor necrosis factor alpha-, and Mycobacterium tuberculosis-induced pro-inflammatory signals. *J Biol Chem* 280(48)**,** 39914-39924. doi: 10.1074/jbc.M502820200.

Wilson, K.P., Black, J.A., Thomson, J.A., Kim, E.E., Griffith, J.P., Navia, M.A., et al. (1994). Structure and mechanism of interleukin-1 beta converting enzyme. *Nature* 370(6487)**,** 270-275. doi: 10.1038/370270a0.

Wise, C.A., Gillum, J.D., Seidman, C.E., Lindor, N.M., Veile, R., Bashiardes, S., et al. (2002). Mutations in CD2BP1 disrupt binding to PTP PEST and are responsible for PAPA syndrome, an autoinflammatory disorder. *Hum Mol Genet* 11(8)**,** 961-969.

Xie, X., Gu, Y., Fox, T., Coll, J.T., Fleming, M.A., Markland, W., et al. (1998). Crystal structure of JNK3: a kinase implicated in neuronal apoptosis. *Structure* 6(8)**,** 983-991.

XOMA (2013). "XOMA Announces Encouraging Interim Results From Gevokizumab Phase 2 Study for Moderate to Severe Acne Vulgaris". (http://investors.xoma.com/releasedetail.cfm?ReleaseID=731711).

Yamamoto, M., Sato, S., Hemmi, H., Uematsu, S., Hoshino, K., Kaisho, T., et al. (2003). TRAM is specifically involved in the Toll-like receptor 4-mediated MyD88-independent signaling pathway. *Nat Immunol* 4(11)**,** 1144-1150. doi: 10.1038/ni986.

Yamamoto, M., Sato, S., Mori, K., Hoshino, K., Takeuchi, O., Takeda, K., et al. (2002). Cutting edge: a novel Toll/IL-1 receptor domain-containing adapter that preferentially activates the IFN-beta promoter in the Toll-like receptor signaling. *J Immunol* 169(12)**,** 6668-6672.

Yamasaki, S., Ishikawa, E., Sakuma, M., Hara, H., Ogata, K., and Saito, T. (2008). Mincle is an ITAM-coupled activating receptor that senses damaged cells. *Nat Immunol* 9(10)**,** 1179-1188. doi: 10.1038/ni.1651.

Yang, D.D., Conze, D., Whitmarsh, A.J., Barrett, T., Davis, R.J., Rincon, M., et al. (1998). Differentiation of CD4+ T cells to Th1 cells requires MAP kinase JNK2. *Immunity* 9(4)**,** 575-585.

Yarovinsky, F. (2014). Innate immunity to Toxoplasma gondii infection. *Nat Rev Immunol* 14(2)**,** 109-121. doi: 10.1038/nri3598.

Yoon, S., and Seger, R. (2006). The extracellular signal-regulated kinase: multiple substrates regulate diverse cellular functions. *Growth Factors* 24(1)**,** 21-44. doi: 10.1080/02699050500284218.

Yu, N., Liu, S., Yi, X., Zhang, S., and Ding, Y. (2015). Serum amyloid A induces interleukin-1beta secretion from keratinocytes via the NACHT, LRR and PYD domains-containing protein 3 inflammasome. *Clin Exp Immunol* 179(2)**,** 344-353. doi: 10.1111/cei.12458.

Yu, X.J., Li, C.Y., Dai, H.Y., Cai, D.X., Wang, K.Y., Xu, Y.H., et al. (2007). Expression and localization of the activated mitogen-activated protein kinase in lesional psoriatic skin. *Exp Mol Pathol* 83(3)**,** 413-418. doi: 10.1016/j.yexmp.2007.05.002.

Zan, Y., Haag, J.D., Chen, K.S., Shepel, L.A., Wigington, D., Wang, Y.R., et al. (2003). Production of knockout rats using ENU mutagenesis and a yeast-based screening assay. *Nat Biotechnol* 21(6)**,** 645-651. doi: 10.1038/nbt830.

Zarubin, T., and Han, J. (2005). Activation and signaling of the p38 MAP kinase pathway. *Cell Res* 15(1)**,** 11-18. doi: 10.1038/sj.cr.7290257.

Zeke, A., Misheva, M., Remenyi, A., and Bogoyevitch, M.A. (2016). JNK Signaling: Regulation and Functions Based on Complex Protein-Protein Partnerships. *Microbiol Mol Biol Rev* 80(3)**,** 793-835. doi: 10.1128/MMBR.00043-14.

Zelensky, A.N., and Gready, J.E. (2005). The C-type lectin-like domain superfamily. *FEBS J* 272(24)**,** 6179-6217. doi: 10.1111/j.1742-4658.2005.05031.x.

Zervos, A.S., Faccio, L., Gatto, J.P., Kyriakis, J.M., and Brent, R. (1995). Mxi2, a mitogen-activated protein kinase that recognizes and phosphorylates Max protein. *Proc Natl Acad Sci U S A* 92(23)**,** 10531-10534.

Zhai, Z., Liu, W., Kaur, M., Luo, Y., Domenico, J., Samson, J.M., et al. (2017). NLRP1 promotes tumor growth by enhancing inflammasome activation and suppressing apoptosis in metastatic melanoma. *Oncogene*. doi: 10.1038/onc.2017.26.

Zhao, Y., Yang, J., Shi, J., Gong, Y.N., Lu, Q., Xu, H., et al. (2011). The NLRC4 inflammasome receptors for bacterial flagellin and type III secretion apparatus. *Nature* 477(7366)**,** 596-600. doi: 10.1038/nature10510.

Zhong, F.L., Mamai, O., Sborgi, L., Boussofara, L., Hopkins, R., Robinson, K., et al. (2016). Germline NLRP1 Mutations Cause Skin Inflammatory and Cancer Susceptibility Syndromes via Inflammasome Activation. *Cell* 167(1)**,** 187-202 e117. doi: 10.1016/j.cell.2016.09.001.

Zhou, R., Yazdi, A.S., Menu, P., and Tschopp, J. (2011). A role for mitochondria in NLRP3 inflammasome activation. *Nature* 469(7329)**,** 221-225. doi: 10.1038/nature09663.

Zihni, C., Mills, C., Matter, K., and Balda, M.S. (2016). Tight junctions: from simple barriers to multifunctional molecular gates. *Nat Rev Mol Cell Biol* 17(9)**,** 564-580. doi: 10.1038/nrm.2016.80.

Zook, E.C., and Kee, B.L. (2016). Development of innate lymphoid cells. *Nat Immunol* 17(7)**,** 775-782. doi: 10.1038/ni.3481.

APPENDIX

ABBREVIATIONS

AIM2	absent in melanoma 2
A/A	antibiotic-antimycotic
Ala	alanine
ALR	AIM2-like receptors
AMP	antimicrobial peptide
ANOVA	analysis of variance
AP	alkaline phosphatase
APC	antigen-presenting cell
APS	ammonium persulfate
ASC	apoptosis-associated speck-like protein containing a CARD
ATP	adenosine triphosphate
BMM	bone marrow-derived macrophage
Ca^{2+}	calcium ion
CAPS	cryopyrin-associated periodic syndrome
CARD	caspase-recruitment domain
CD	cluster of differentiation
CHS	contact hypersensitivity
CL	cell lysate
CLR	C-type lectin receptor
CoIP	co-immunoprecipitation
COP	CARD-only protein
COX	cyclooxygenase
CR-HPK	CRISPR/Cas9-targeted HPK
CRISPR	clustered regularly interspaced short palindromic repeats
DAMP	danger-associated molecular pattern
DC	dendritic cell
ddH_2O	bidistilled water
DIRA	deficiency of IL-1 receptor antagonist
DM	differentiation medium
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
Doxy	doxycycline
dsDNA	double stranded DNA
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay

ERK	extracellular signal-regulated kinase
FCAS	familial cold autoinflammatory syndrome
FMF	familial Mediterranean fever
Glu	glutamic acid
GSDMD	gasdermin D
HA	human influenza hemagglutinin
HMGB1	high mobility group box-1
HPK	human primary keratinocyte
HRR	homologous recombination repair
IFN	interferon
Ig	immunoglobulin
IL-1	inteleukin-1
IL-1R	interleukin-1 receptor
IL-1Ra	IL-1R antagonist
ILC	innate lymphoid cell
IP	immunoprecipitation
IRAK	IL-1R-activating kinase
JNK	Jun N-terminal kinase
K^+	potassium ion
ko	knockout
LC	Langerhans cell
LDH	lactate dehydrogenase
LPS	lipopolysaccharide
LRR	leucine-rich repeat
M-CSF	macrophage colony-stimulating factor
MAP2K	MAPK kinase
MAP3K	MAPK kinase kinase
MAPK	mitogen-activated protein kinase
MAS	macrophage activation syndrome
MAVS	mitochondrial antiviral-signaling protein
MBP	mannose-binding protein
MDP	muramyl dipeptide
MHC	major histocompatibility complex
MKD	mevalonate kinase deficiency
MNK	MAPK interacting kinase
mRNA	messenger RNA

MSK	mitogen- and stress- activated kinases
MSU	monosodium urate
MWS	Muckle-Wells syndrome
Mϕ	macrophage
NAIP	NLR family, apoptosis inhibitory protein
NBD	nucleotide-binding domain
NBT	nitro blue tetrazolium
NET	neutrophil extracellular trap
NF-kB	nuclear factor kappa B
NHEJ	non-homologous end joining
NK	natural killer
NLR	NOD-like receptor
NLRC	NLR CARD-containing protein
NLRP	NLR PYD-containing protein
NOD	nucleotide-binding oligomerization domain
NOMID	neonatal-onset multisystem inflammatory disease
ns	not significant
PAM	protospacer-adjacent motif
PAMP	pathogen-associated molecular pattern
PAPA	pyogenic arthritis, pyoderma gangrenosum, and acne
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
PEC	peritoneal exudate cell
PFA	paraformaldehyde
PKC	protein kinase C
POP	PYD-only protein
Pro	proline
PRR	pattern recognition receptor
Puro	puromycin
PYD	pyrin domain
qPCR	quantitative PCR
qRT-PCR	quantitative real-time PCR
RA	rheumatoid arthritis
RIG	retinoic acid-inducible gene
RLR	RIG-I-like receptor
RNA	ribonucleic acid

ROS	reactive oxygen species
RT	room temperature
SAPK	stress-activated protein kinase
SDS	sodium dodecyl sulphate
SFM	serum-free medium
sgRNA	single-guide RNA
shRNA	short hairpin RNA
siRNA	small interfering RNA
SN	supernatant
ssDNA	single stranded DNA
T3SS	type 3 secretion system
TAK1	TGF-β-activated kinase 1
TALEN	Transcription Activator-Like Effectors nuclease
TGF	transforming growth factor
Thr	threonine
TIF	Triton X-100 insoluble fraction
TIR	Toll/interleukin-1 receptor
TLR	Toll-like receptor
TNF	tumor necrosis factor
TRAF	TNF receptor-associated factor
TSF	Triton X-100 soluble fraction
Tyr	tyrosine
UV	ultraviolet light
v/v	volume per volume
v/w	volume per weight
VSV	vesicular stomatitis virus
WB	Western blot
wt	wild-type
ZFN	zing-finger nuclease

UNITS

bp	base pairs
°C	degree Celsius
cm	centimetre
Da	Dalton
kDa	kiloDalton
dpi	dots per inch
g	gram
µg	microgram
mg	milligram
ng	nanogram
pg	picogram
h	hour
hr	hr
J	joule
kJ	kilojoule
mJ	millijoule
l	liter
µl	microliter
ml	milliliter
m	meter
µm	micrometer
mm	millimeter
nm	manometer
min	minute
M	molar
µM	micromolar
mM	millimolar
nM	nanomolar
OD	optical density
s	second
V	volt
kV	kilovolt
W	watt

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CURRICULUM VITAE

Name	**Gabriele Fenini**
Academic title	MSc ETH in Biology
Date of birth	26.05.1986
Place of birth	Sorengo, TI
Nationality	Swiss
Position	PhD student
Address	Farman-Strasse 2
	8152 Glattpark (Opfikon), ZH
Email	gabriele.fenini@usz.ch
	gabriele@gfenini.ch

Education

01.2013-present	**PhD**
	MIM PhD program, Life Science Zurich Graduate School
	Univeristy of Zurich
01.2011-06.2012	**MSc ETH in Biology**
	Major in Cell Biology
	Swiss Federal Institute of Technology Zurich (ETHZ)
09.2006-03.2011	**BSc ETH in Biology**
	Swiss Federal Institute of Technology Zurich (ETHZ)
09.2001-06.2005	Maturità liceale cantonale scientifica – Biologia e Chimica
	Liceo Cantonale Lugano 2, Savosa TI

Research experience

01.2013-08.2017	**"The role of mitogen-activated protein kinases in inflammasome activation"**
	PhD Thesis, Research group Prof. Dr. Lars E. French
	Dermatology Research, University Hospital Zurich
12.2011-06.2012	**"Role of scaffold protein 14-3-3θ in modulation of inflammasome activity"**
	Master Thesis, Research group Prof. Dr. Lars E. French
	Dermatology Research, University Hospital Zurich

Grants

2013-2015	F**orschungskredit Candoc** (FK-13-027), University of Zurich

Scientific publication

2017	The p38 mitogen-activated protein kinase critically regulates human keratinocyte inflammasome activation. **G. Fenini**, S. Grossi, S. Gehrke, T.K. Satoh, H.D. Beer, E. Contassot, and L.E. French. Manuscript submitted to J Invest Dermatol. *In revision*.
2017	Potential of IL-1, IL-18 and Inflammasome Inhibition for the Treatment of Inflammatory Skin Diseases. **G. Fenini**, E. Contassot, and L.E. French. Front Pharmacol 8 (2017) 278. *Review*.
2017	Opposing effects of Nrf2 and Nrf2-activating compounds on the NLRP3 inflammasome independent of Nrf2-mediated gene expression. M. Garstkiewicz, G.E. Strittmatter, S. Grossi, J. Sand, **G. Fenini**, S. Werner, L.E. French, and H.D. Beer. Eur J Immunol 47 (2017) 806-817.
2016	Tumour hypoxia promotes melanoma growth and metastasis via High Mobility Group Box-1 and M2-like macrophages. R. Huber, B. Meier, A. Otsuka, **G. Fenini**, T. Satoh, S. Gehrke, D. Widmer, M.P. Levesque, J. Mangana, K. Kerl, C. Gebhardt, H. Fujii, C. Nakashima, Y. Nonomura, K. Kabashima, R. Dummer, E. Contassot, and L.E. French. Sci Rep 6 (2016) 29914.
2014	IL-1beta drives inflammatory responses to propionibacterium acnes in vitro and in vivo. M. Kistowska, S. Gehrke, D. Jankovic, K. Kerl, A. Fettelschoss, L. Feldmeyer, **G. Fenini**, A. Kolios, A. Navarini, R. Ganceviciene, J. Schauber, E. Contassot, and L.E. French. J Invest Dermatol 134 (2014) 677-85.
2014	Malassezia yeasts activate the NLRP3 inflammasome in antigen-presenting cells via Syk-kinase signalling. M. Kistowska, **G. Fenini**, D. Jankovic, L. Feldmeyer, K. Kerl, P. Bosshard, E. Contassot, and L.E. French, Exp Dermatol 23 (2014) 884-9.
2014	Metastatic melanoma cell lines do not secrete IL-1beta but promote IL-1beta production from macrophages. S. Gehrke, A. Otsuka, R. Huber, B. Meier, M. Kistowska, **G. Fenini**, P. Cheng, R. Dummer, K. Kerl, E. Contassot, and L.E. French, J Dermatol Sci 74 (2014) 167-9.